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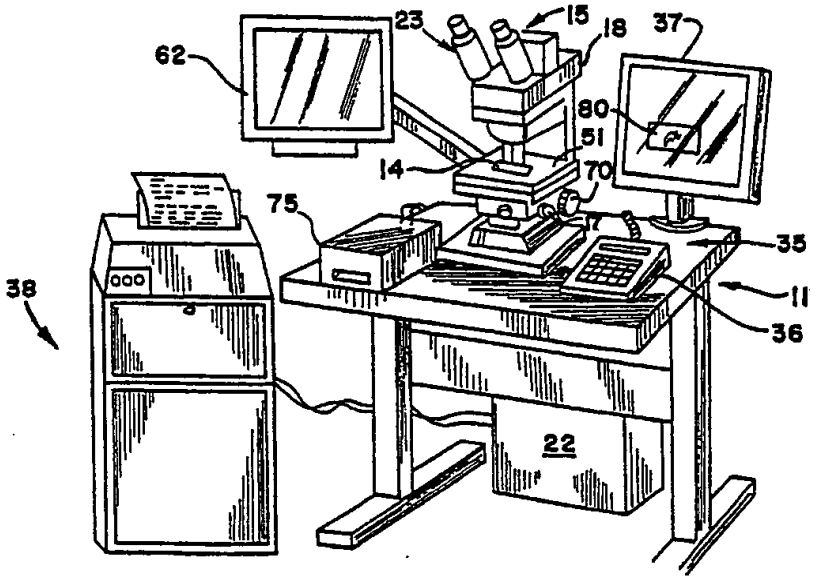

(57) Abstract

A measurement method and apparatus (11) which can acquire quantitative data concerning a plurality of individual cells very quickly by an interactive process through a keyboard (36). The apparatus (11) provides a means for displaying (37) an image (80) of a group of cells from a field on a slide (14) which is magnified by a microscope (15). The image (80) is further digitized and stored in a memory of the apparatus (11). From the digitized image a processor means (22) identifies each possible cell object automatically by a pattern recognition technique. An interactive program utilizing a monitor (62) allows the operator to point to each object or cell of the image (80) in succession and make decisions for classification and quantitative measurements concerning each.

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ANALYSIS METHOD AND APPARATUS FOR BIOLOGICAL SPECIMENS

CROSS REFERENCE TO RELATED APPLICATIONS

The present application is a continuation-in-part application of application Serial No. 794,937 filed November 4, 1985 in the name of James W. Bacus and entitled "Cell Analysis Apparatus and Method with Calibration and Control Slide" which is commonly assigned with the present application. The disclosure of Bacus is hereby expressly incorporated by reference herein.

FIELD OF THE INVENTION

The invention relates generally to the measurement of cell object features and parameters by image analysis, and is more particularly directed to quantitative measurement methods and apparatus for DNA analysis in small cell populations.

BACKGROUND OF THE INVENTION

The present invention is directed to a quantitative testing apparatus and method which may be used for a wide range of diagnostic and prognostic evaluations of various cells, antigens, or other biological materials taken from the human body. However, for purposes of illustration and ease of understanding, the invention will be disclosed in conjunction with its preferred use, which is the quantitative measurement of cellular DNA for the purpose of cancer diagnosis and prognosis. More specifically, the present invention is directed to a method of interactive image analysis for analyzing and quantifying the DNA in specimen cells taken from a person.

The current state of the art in the pathology laboratory is to measure the DNA content of a cell by the visual observation of the pathologist who observes

primarily the shape and texture of suspected cancer cells and who then classifies the cells into a normal category or into one of several abnormal cancer categories. However, such evaluations are very subjective and can not differentiate and quantify small changes in DNA within individual cells or in very small populations of abnormal cells. These small changes may represent an incipient stage of cancer or a change in cell structure due to treatment of the cancer by chemotherapy or radiation. Such small changes are, therefore, important in the diagnosis and prognosis of these diseases.

However, the advantage in diagnosis and/or prognosis of abnormal ploidy distributions that a pathologist viewing a specimen under a microscope has is the discerning expertise of a skilled person in classifying cells as normal or abnormal. There is an innate human ability to make relatively quick infinite gradations of classification, i.e., almost normal, slightly abnormal, etc. On the other hand, the classification and measurement of cell features and parameters by a pathologist on a cell-by-cell basis is extremely tedious and time consuming. Broad statistical analysis of such cell data taken by hand is relatively difficult because each record has to be entered and then processed. For different records, taken at different times, and under varying conditions broad statistical categorizations may be unreliable.

The alternative is automated cell analysis where the pathologist uses specialized equipment to perform the analysis. In automatic cell analysis, such as that accomplished by a flow cytometer, mass tests are performed in gross on a specimen cell population without a researcher being able to exclude or include certain data of the population. The specimen is measured "as is" without really knowing what cells are being measured and how many. Important single cell data or data from

relatively small groups of cells are lost in the overall averaging of a specimen. Further, relatively large amounts of a specimen have to be used to provide any accuracy because of the averaging problem. This was considered necessary in the prior art to process large amounts of cell data relatively quickly so that the results will be fairly accurate. Again small changes in individual cells or small cell populations cannot be discerned.

Although there are commercially available general purpose flow cytometers, they are very expensive and can handle only liquid blood specimens or tissue disaggregations. These cytometers are incapable of working on standard tissue sections or using conventional microscope slides which are the preferred specimen forms of pathology laboratories. Additionally, a flow cytometer does not allow for the analysis of morphological features of cells such texture, size and shape of cell nuclei and alterations in the nuclear-to-cytoplasmic ratios of cells.

Moreover, for such cell analysis, either automatic or manual, to be of real value there should be some way of verifying the results. The normal scientific method for accomplishing verification is to save the specimen so that another pathologist can compare his analysis to that of the first. However, for individual cells classified by manual means this indicates either photographs, drawings, or other imprecise mediums because it is extremely difficult to fix a tissue specimen for a long period of time. Further, even with those techniques where such specimens are fixed sufficiently for subsequent viewing, there remains the problem of finding the same cell or small population of cells from which an original evaluation was made and presenting the same conditions for viewing. With automated methods, the sample is consumed and verification can only occur by observing similar tissue from the same area.

SUMMARY OF THE INVENTION

The invention solves these and other problems relating to the image analysis of various features and parameters of cell objects by providing a measurement method and apparatus which can acquire quantitative data concerning a plurality of individual cells very quickly by an interactive process with a pathologist or an operator. The apparatus provides means for displaying an image of a group of cells from a field of a microscope slide. The image is further digitized and stored in a memory of the apparatus. From the digitized image a processor means identifies each possible cell object automatically by pattern recognition technique. An interactive program allows the operator to point to each object or cell in succession and make decisions for classification and measurements concerning each. For quantitative DNA analysis, the measurement is of the optical density of the cell object and the classification is by a pathologist as to whether the cell appears normal or cancerous. The decisions can include whether to accept or reject the particular cell for further processing. The cell object, if selected, can also be classified into one of several classifications for later statistical analysis. The apparatus further has means which provide for the classification and storing of more than one image.

One of the features the present apparatus provides is the enhancement of the image by providing a threshold value for the data prior to its display. The displayed image corresponds to a plurality of pixels which form the digitization of the image field. Below such threshold, a pixel in the display is shown as white or the absence of any information. A grey scale image above the threshold is displayed for the remaining pixels. This feature advantageously reduces background clutter and enhances the visual characteristics of the cell objects in the field. The threshold is variable

and can be used to mask certain features while enhancing others. The full scale of grey resolution is used above the threshold for excellent contrast differentiation.

Another feature of the invention provides for the verification of the measured data. When each image or field is digitized and stored, a reference number is stored with the data. Preferably this reference number is the relative X, Y position of the image from a selected coordinate origin on a slide. The invention provides means for displaying the actual position of the area of a slide being viewed. Therefore, to verify a previously stored image, a slide is remounted with respect to a reference and positioned until its actual displayed position matches the stored reference position. Thus, the data and analysis of the study can be verified not only by the data images from a slide, but the actual slide image used in the analysis can be found readily.

When the apparatus is used for DNA analysis, tissue and cell specimens are applied to a slide which is then stained with a specific stain that combines proportionately with the DNA and essentially renders invisible the remainder of the cell so that the image analysis can measure the optical density of the DNA which is concentrated in the nucleus of the cell. The stain associates with the DNA to provide a detailed nuclear structure and pattern which may be visually observed and interpreted by the pathologist using the apparatus for classification. The amount of DNA in the malignant cells is substantially greater than that for normal cells because the malignant cells are usually dividing and replicating rapidly or the malignant cells have abnormal numbers of chromosomes or have defective chromosomes.

The preferred and illustrated apparatus of the present invention can not only detect minute alterations in the nucleus by providing a real and accurate

measurement of the DNA mass in picograms but also can measure and quantify the amount of DNA and relate it to stored statistical analyses to aid in the diagnosis. More specifically, the invention allows an iterative analysis of specimen population cells and provides a histogram or other statistical display of the population distribution of the cells with respect to their DNA content and with respect to a standard DNA for normal cells so that subtle shifts in population distribution can be readily understood. To this end cell nuclei images are not only acquired and stored but the data therefrom can be integrated with other statistical data to provide multivariate analysis, discrimination of cells, histograms, and scattergrams of cells or cell populations.

The use of image analysis techniques and equipment for stained specimens by pathologists in a conventional pathology laboratory involves solving a number of problems which have been overcome by the present invention. For example, while there are a number of available staining techniques which can be used, such as an Azure A Feulgen staining technique described hereinafter, the staining of the DNA will vary substantially not only from slide to slide and from batch to batch by the same pathologist but also will vary substantially between different pathologists and different laboratories. Because the present image analysis apparatus measures grey level or optical density and because it is desired to provide a true actual measurement of DNA in picograms, it is important to overcome the problem of different staining factors for different specimens. Also, image analysis techniques which use adjustable microscopes and optical lighting provide different intensities of light when used by the pathologist. Trained researchers, in research laboratories may be equipped to adjust the optical intensity to the desired conditions for image

analysis by image pattern techniques but this generally cannot be accomplished with the precision necessary in the usual pathology laboratory. Thus, there is a need to overcome the problem of this light intensity and, therefore, optical density variable.

Additionally, the present invention is directed to overcoming the problem of high costs heretofore associated with automated equipment used for image analysis; and to this end, the present invention provides a facile interactive system in which the pathologist performs a number of tasks and performs the preparation of cells and their selection by manipulation of the equipment. The pathologist also is provided with slides which are specially prepared and calibrated with reference cells to aid in the analysis of the specimen cells and to assist in overcoming the above-described staining problem.

The present invention has particularly been developed to locate cells for examination as to their morphology and to preserve their location for a later or corroborating analysis by a second pathologist when so desired. As will be explained, with respect to nuclei, measurements may be obtained as to their area in microns, total nuclear optical density or nuclear mass in picograms, average nuclear optical density, nuclear texture, and deviation of the nuclear shape from being a round nucleus.

Accordingly, a general object of the invention is to provide a new and improved method and apparatus for analyzing cells or other biological materials by using image analysis techniques.

Another object of the invention is to provide a new and improved method and apparatus for making a quantitative ploidy analysis of cells using image pattern recognition equipment.

A further object of the invention is to provide a new and improved slide or support for specimen cells

having control cells or cell objects thereon which are used for calibrating the image analysis equipment.

These and other objects, features, and aspects of the invention will become apparent upon reading the following detailed description when taken in conjunction with the attached drawings wherein:

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a pictorial representation of an image analysis system constructed in accordance with the invention;

FIG. 2 is a functional block diagram of the image analysis system illustrated in FIG. 1 which is adapted to perform an image analysis method in accordance with the invention;

FIG. 2A is a functional system diagram illustrating the major operations of the system control illustrated in FIG. 2;

FIG. 3 is an electrical block diagram of an interface electronics for the X, Y position circuitry illustrated in FIG. 2;

FIG. 4 are representative timing waveforms for the input to the interface electronics illustrated in FIG. 2;

FIGS. 5A, 5B, and 5C are perspective, top, and cross sectional views, respectively, of a slide particularly adapted for use in the image analysis system illustrated in FIG. 1 having separate areas for calibration cell objects and specimen cell objects;

FIGS. 6-9 are pictorial representations of histograms for different normal and abnormal cell populations;

FIG. 10 is a pictorial representation of the different screen images viewable on the instruction monitor illustrated in FIG. 1;

FIG. 11 is a pictorial representation of the main screen image which appears on the instruction monitor illustrated in FIG. 1;

FIG. 12 is a pictorial representation of the calibration image screen which appears on the instruction monitor illustrated in FIG. 1;

FIG. 13 is a pictorial representation of the analysis screen image which appears on the instruction monitor illustrated in FIG. 1;

FIG. 14 is a pictorial representation of the X, Y field coordinates image screen which appears on the instruction monitor illustrated in FIG. 1;

FIG. 15 is a pictorial representation of the option list for the main screen illustrated in FIG. 11;

FIG. 16 is a pictorial representation of the option list for the calibration screen illustrated in FIG. 12;

FIG. 17 is a pictorial representation of the option list for the analysis screen illustrated in FIG. 13;

FIG. 18 is a pictorial representation of the measure operations for the calibration screen illustrated in FIG. 12;

FIG. 19 is a pictorial representation of the analysis operations for the analysis screen illustrated in FIG. 13;

FIG. 20 is a pictorial representation in time sequence of the analysis operation for a field of cell objects as seen on the image display illustrated in FIG. 1;

FIG. 21 is a pictorial diagram of a slide used for image analysis which has been divided into a plurality of selectable image fields by the image analysis system illustrated in FIG. 2;

FIG. 22 is a pictorial diagram of a histogram used for light source calibration in the image analysis system illustrated in FIG. 2;

FIG. 23 is a flow chart diagram of the program which reads the X, Y coordinate position of the platform of the microscope illustrated in FIG. 1; and

FIG. 24 is a software flow chart of the program whose function is to process the analysis and measurement operations for the analysis and measurement screens illustrated in FIGS. 12 and 13, respectively.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The apparatus illustrated in FIGS. 1 and 2 can be used to develop histograms, and other statistical data, of cell populations for the diagnosis and prognosis of malignancies and other diseases. To illustrate the utility of such statistical analysis reference is directed to FIGS. 6-9.

Referring now to FIG. 6 there is shown a normal ploidy histogram having cell number versus mass distribution for healthy, non-dividing cells. The number of cells is provided on the ordinate axis and their nuclear mass on the abscissa. If the cell population shown in the figure is not dividing, the DNA content should be peaked around a normal peak G0/G1 which is the diploid amount, 7.18 picograms/cell. This relative mass of DNA is labelled as 1.0 to normalize the abscissa of the histogram. FIG. 7 also shows a normal cell population which is dividing, where there is a significant G0/G1 peak at 1.0 and a second peak G2 at 2.0. The peak at 2.0 is normal because some of the cells are in division and have double the normal diploid amount of DNA. The saddle S between the two peaks is relatively low and does not indicate any malignancy.

Comparing the histogram in FIG. 8 with the first two, it is seen that this cell population is skewed from normal having a higher first peak around 1.5 and a second peak around 3.0. Further, the saddle S is more pronounced and can be rough in cell count. This histogram may show a malignancy because of the abnormally high DNA content of many of the cells. This high DNA content is likely indicative of the increased ploidy amount of malignant cells which are rapidly

dividing. Likewise, in FIG. 9 it is shown that the G0/G1 peak occurs at 1.0 with a normal diploid amount of DNA but has a relatively large trailing saddle from 1.0 to 2.8. A normal G2 second peak is not noted and is indicative of an abnormal cell population. The shape of the histogram is likely due to abnormal DNA amounts in cells and clones of cells indicative of malignancy. Therefore, from the shapes and changes in cell distribution histograms, diagnostic and prognostic information can be obtained.

In the implementation shown, the system is a computerized image analysis system designed to measure a number of cell object features and parameters from their image on a typical glass slide. The instrument includes a sophisticated digital image processing system controlled by software to perform quantitative analysis on individual cells for nuclear DNA content by Feulgen staining as well as measurement of other nuclear features. The imaging system couples the ability of a pathologist to identify cells to be studied with the capability of computer enhanced, high resolution digital video image processing to quantify optical and stain density accurately.

In general, a pathologist first prepares a touch preparation or a needle aspirate of fresh tissue. Alternatively, embedded samples can be visually inspected for areas of interest and then deparaffinized and disaggregated by mincing in the presence of pepsin to produce a single cell suspension that is then placed on a microscope slide. After fixation and staining with Azure A Feulgen stain, the preparation is ready for analysis.

The operator has the option of classifying the cells morphologically into any one of six categories or rejecting inappropriate cells or debris. The cell data are processed by a system control and the cellular elements are characterized by a quantitative DNA

analysis for each cell class. The information when compared with either a standard cell calibration or published data allows a pathologist to accurately quantify and classify abnormalities that might ordinarily be described only verbally from the image a person sees.

The addition of quantitative data enables pathologists to perform their work in a more standardized and reproducible manner. The system is of value in classifying lesions that may be malignant and in providing prognostic information for known malignancies based on DNA content. The image identification system is more advantageous than common flow cytometry methods of evaluating DNA content. Flow cytometry permits an operator to classify neoplastic cells based only on cell markers. The pathologist, however, never sees the cells that the instrument has examined. In addition, the cell preparation must be used in a short time frame and is consumed in the course of the study. Although a permanent section of a tumor under study may be examined at the same time, there is no guarantee that the same cells are examined in both areas. Also the quantity of tumor available may not be large enough to permit a flow cytometric examination.

In the invention, the quantitative DNA analysis is performed rapidly for the measurement of DNA and ploidy distribution pattern in a cell population under study. The pathologist advantageously selects the cells which are to be used in the population measurements. The measurement of DNA content is useful and believed to be relevant in diagnosing and determining prognosis for a variety of tumors that involve the breast, colorectum, and prostate. The system takes advantage of the pathologists skill to identify visually abnormal cells and then uses a computer aided imaging analysis to analyze quantitatively those particular cells for the parameters desired. Such instrument extends and

augments the recognition and diagnostic skills of the pathologist.

As shown more specifically in the drawings for purposes of illustration, the invention is embodied in a method and apparatus for automatically analyzing "cell objects" which term is used herein to be generic to cells, such as blood cells or cells taken from tumors or the like, which are to be analyzed for the DNA content. The term is also meant to encompass non-biological objects, such as conventional plastic or glass spheres used in biological studies, painted cell images on a slide, or antigens or monoclonal antibodies on cells. The system further finds use where a monoclonal antibody is conjugated with a stain, wherein the stain may be a fluorescent material which is excited at one wavelength and which can then be analyzed at another wavelength where fluorescence occurs. By way of example, the present invention is useful for ploidy analysis, red blood cell analysis, pap smear cell analysis, monoclonal antibody analysis, and the analysis of other infectious diseases which can be diagnosed by DNA probes for viruses. The imaging and analysis system is, therefore, advantageously used in a number of studies where one of the optical characteristics, such as optical density, of a cell object may be used to determine some parameter or feature of that object which is diagnostic or prognostic for a particular condition.

As shown in FIGS. 1 and 2 of the drawings, the invention is embodied as an apparatus 11 which functionally operates as a digital image processing system 13 (FIG. 2). The apparatus 11 comprises a high resolution microscope 15 with which an operator can view specimens on a support, in the preferred embodiment a glass slide 14. The microscope 15 has means 70 for focusing its optics on the slide and a platform 51 movable incrementally by means 11 and 17 in X and Y directions for viewing various areas thereof. The

specimens or material on the slide 14 are further viewable by the imaging system 13 which is controlled by a system control 22 in the form of a digital processor such as a personal computer. An operator can communicate with the system control 22 via a keyboard 36 and interacts with the apparatus 11 by viewing two displays. A first display, image display 62, is a RGB monitor which displays through the system control 22 the same image as seen through the microscope 15. A second display, instruction monitor 62, is another RGB monitor and is used to provide the operator with interactive prompts, messages, information, and instructions from the program controlling the system control 22. A printer 38 is provided to produce a reliable hard copy output of data produced by the apparatus 11.

The functional schematic of the apparatus 11 is illustrated in FIG. 2 as image processing system 13. The image processing system 13 is used to analyze a plurality of cell objects on the support or glass slide 14 of the microscope 15. Suitable high resolution microscope optics 16 receive light from a variable intensity source 17 through the slide 14. The optics 16 form an optical image of each of the cell objects on the slide 14 and transmit them to a image splitter 25 which can take the form of a prism.

On one side of the splitter 25, a television camera 18, or other detector, converts the optical images point by point into a scanned electronic signal representing the optical intensity of each point in the image. The output of the camera 18 which is a standard NTSC analog video signal is applied to an analog to digital converter of an image processing interface 21. The image processing interface 21 converts the image signal from the television camera 18 to a digitized signal which is received and stored by the system control 22. Because of the continuous scanning, a real

time image of the area the optics 16 are focused on is provided by the image display 37. In general, the image is a 512 X 512 array of pixels each having a measured light intensity.

On the other side of the image splitter 25 is located the viewing optics 23 of the microscope 15. This parafocal arrangement allows the same image in the viewing optics 23 to be displayed on the image display 37. This feature allows the positioning of the platform 51 by the manual X, Y adjustment means 11 and 17 until the operator views a field of interest on the slide 14. At that time, a computer enhanced digitized image of the selected field is displayed on the image display 37 for further analysis.

Both of the displays 37 and 62 are controlled by the system control 22 through standard video interface circuitry 39 and 61, respectively. Similarly, the keyboard 36 and printer 38 communicate with the system control 22 through conventional interface circuitry 35 and 41, respectively. In addition, the system control 22 controls a random access memory 73 and bulk storage in the form of floppy and hard disk drives 75 through a memory control 71.

All of the interface circuits 21, 35, 39, 41, 61, 71, and 106 can be selectively embodied on printed circuit boards which are mounted in the backplane or card connector of a conventional personal computer forming the system control 22. Preferably, the personal computer can be one manufactured by the IBM Corporation having a model designation AT. Such system control can be run under a disk operating system such as PC DOS version 3.1 or later. The system software for the image analysis is called as an application program from the disk drive 75, and could, for example, be supplied on a floppy disk 77. The system software is read from disk 77 and loaded into RAM 73. After loading program control is transferred to the analysis software to

regulate the various hardware elements previously set forth in a known manner.

The analysis software can be for DNA analysis as hereinafter described or could include other software for various purposes. For example, the analysis of the shape and size as well as hemoglobin content of the cells when examining red blood cells may be accomplished in accordance with the pattern recognition technique and analysis method disclosed in U.S. Patent Nos. 4,097,845 and 4,199,748 issued to Bacus which are hereby incorporated by reference as if fully reproduced herein.

FIG. 2A illustrates functional operation of the system software where control logic for the instrument hardware, the image display, and the instruction display are used to perform the main system functions. The main system functions are patient labelling, light level calibration, control cell calibration, cell data acquisition, cell classification, cell analysis, and report generation.

The interface circuitry, except the interface electronics 106, can be standard control circuits for the various functions illustrated. The interface electronic 106 is a specialized circuit which is more fully illustrated in FIGS. 3 and 4. The X, Y position sensors allow the field under analysis to be given a position.

Herein the X and Y locations of each field are easily determined for any given location by a novel method and apparatus which includes, as best seen in FIGURE 2, an X direction sensing strip 100 which may be fastened to the underside of the microscope stage 51 for movement with the stage 51 past a sensing pad 102, which is secured to a stationary part of the microscope. The sensor provides an analog output to an interface electronics 106 which provides the X coordinate in digital numbers to the system control 22 for storing in

memory and for displaying on the monitor screen 62. Likewise, a similar strip 110 is fastened to the stage 51 for movement in the Y direction with the stage past a sensing pad 112 which is secured to a stationary part of the microscope is so that the pad may provide an analog signal to the interface electronics 106 which supplies digital signals to the instrument control logic 122 for storage of the Y coordinate and for showing the Y coordinate on the video monitor adjacent the X coordinate. Manifestly, the system can be reversed with the sense heads fastened to the stage for movement therewith and with the sensor strips 100 and 110 being mounted stationary to provide analog signals as the heads move thereacross.

The circuitry for interfacing with the position sensors is more fully detailed in FIGS. 3 and 4. The position sensors are magnetic sensors having the pair of sensor strips 100 and 102 for the X coordinate and the pair of sensor strips 110 and 112 for the Y coordinate. The sensor strips 100 and 110 are affixed orthogonal to each other such that these strips form a reference axes for the coordinate system. The moveable sensor pads 102 and 112 which are affixed to the moveable base of the microscope 15 travel along the strips 100 and 102 sensing the relative movement between the members depending upon the relative position of the moveable pad with respect to the fixed strip. The sensors are connected to a measuring chip 18 which includes circuitry for measuring the relative position between the strips and for generating a digital numbers based on that sensed parameter. The X sensing strips 100 and 102 are connected to the sense XA and sense XB inputs of the measuring circuit 18 and similarly the Y sensing strips 110 and 112 are connected to the sense YA and sense YB inputs of the circuit. The circuit 18 is an internally timed position to digital number generator which outputs a 3 byte serial digital number depending

upon the relative position of the moveable pad with respect to the fixed strip from outputs OXA and OYA. This 3 byte number is serial and is accompanied by a clock signals XCLK or YCLK for timing purposes.

Each of these bursts of 24 bits are at a frequency of approximately 24 KHz. and occur every 100 milliseconds. The measurement circuit 18, therefore, produces a 3 byte number for the X position every 100 milliseconds and a 3 byte number representative of the Y position every 100 milliseconds. The outputs OXA and OXB are connected respectively to the serial input IN and clock input CLK of a shift register 20. Similarly the output OYA and output OYB of the circuit 18 are connected respectively to the serial input IN and clock input CLK of shift register 22. The signal XCLK will clock or time the 24 bits of the position number into the shift register 20 every 100 milliseconds. This number is then stored in the shift register 20 until it is to be used by the main control program. The Y position of 24 bits is serially presented to the input of the shift register 22 and clocked in to the device by the signal YCLK every 100 milliseconds. The shift registers 20 and 22 act to hold these position numbers between communication bursts from the measurement circuit 18.

The shift registers 20 and 22 are connected to the system control 22 by the address lines A0-A15 of its address bus and by its data lines T0-T7 of the data bus. The 8 bit data bus is connected in parallel to the byte outputs A, B, and C of the shift register 20 and the byte outputs A, B, and C of the shift register 22. Each output represents one of the bytes of a 24 bit position word for either the X or Y position. The outputs are enabled by the input lines A, B, and C connected to the outputs Q0-Q2 of a decoder 24. The address bus lines A0-A15 are connected to the input lines of the decoder 24.

The decoder 24 translates a specific address assigned for each byte of the position words and enables that byte from the respective shift register so that it can be read in on the data bus 28. For example, to read the X position, the system control 22 would output the address which is used by the decoder 24 to enable the A output of the shift register 20 and then read that byte in through the data bus 28. Thereafter, the system control 22 would output the address which would enable the B output of shift register 20, and then read that byte from the data bus 28. Subsequently, the system control 22 will output the address to enable the C output of shift register 20 and read that byte. The operation is identical for reading the Y position word from the shift register 22.

The reading and loading of the shift registers 20 and 22 is entirely asynchronous. Because the positions of the X and Y coordinate are updated every 100 milliseconds, a relatively simple transfer scheme like this can be implemented. If the shift registers 20 and 22 are shifting when the position words are being read by the computer, then the software will make suitable comparisons to determine whether the input number read is a correct number or the shift register was inputting another number when it was read.

A flow chart of the subroutine which reads the shift registers 20 and 22 is more fully illustrated in FIG. 23. The subroutine may be called periodically to determine the actual X, Y position of platform 51. The program begins by reading and storing the X position twice in blocks A225-A231. A comparison is made to see if the two are equal (A=B) and, if not, loops back to the start. A third read and store in blocks A235 and A237 precedes a comparison of A and C in block A239. A negative branch begins the process once again while a positive branch starts the reading of the Y position in the same manner. The technique requires three reads to

match before the number is accepted as the correct position, thereby eliminating the possibility that the table was moved or the shift registers shifted between reads.

Because the apparatus 11 may be used in various offices such as pathology offices having persons of varying degrees of skill and knowledge about image analysis, the microscope light source 17 may be variously adjusted by different operators such that the background may have a different light intensity not only from machine to machine but also at different times depending on the age and nature of the lamp doing the illumination. When the cell objects are DNA nucleus, the stained nuclei appear darker and have high darker gray levels than the cells which have fewer or no DNA content. The particular light intensity level is desired to be known in an accurate and real manner; and hence, it is important that there be a calibration of the light intensity to eliminate errors which might be introduced if differences in light intensity levels are not accounted for.

A further problem with widespread usage of equipment of the foregoing kind is the staining factor by which is meant that the user may be applying either a heavy amount or a light amount of the Azure A stain. This will result in a variation of the gray level being viewed through microscope 15 and by the camera 18 which is then analyzed as to the particular DNA content. Thus, there is a need that the apparatus 11 be calibrated to eliminate differences because of the staining factor so as to provide a true indication of the actual amount of hemoglobin, DNA, or antigens or monoclonal antibodies on cells, etc. being analyzed.

In accordance with the present invention, calibration material 40 (FIG. 5A) is provided on the slide 14 which, when viewed by the operator under a calibration step of the system software allows the

operator to adjust and to calibrate the apparatus prior to the measuring and analyzing of specimen cell objects 12 on the slide 14.

In the illustrated embodiment of the invention there are provided two different calibrating materials on the slide 14 with the first calibrating material being the control cell objects 40 which are stained simultaneously with the staining of the specimen cell objects 12. The simultaneous staining permits the analysis of the control cell objects to be compared to a predetermined stored reference light intensity, gray level, or optical density which the control cell objects 40 have after staining. If the cell objects are stained either too lightly or too heavily, the amount of understaining or overstaining can be quantitatively analyzed and adjusted for as will be described hereinafter.

In the preferred embodiment of the invention, the calibration material also includes an optical density reference material 45 which is usually a printed mark on the slide which has a predetermined known optical density, which can be used as a reference to calibrate the instrument. As will be explained in greater detail hereinafter instructions are provided to the operator from the system software to the operator. Following these instructions, the operator manually adjusts the background light intensity of source 17 until the desired intensity is obtained for the reference material 45. As will be explained hereinafter, this step provides the system control 22 with a calibration to read the proper optical density of the objects on slide 14.

As a safeguard to the integrity of the system, it may be desired to provide an integrity check or identification step for the slide 14 by analyzing a predetermined and prefixed optical pattern on the slide which is read and measured as to gray levels and

physical dimensions before the analyzing may be begun. Herein, the optical integrity pattern may be in the form of initials CAS located above the control cell objects as seen in FIGS. 5A-5C. Manifestly, the integrity check may be the cross 52 or any other shaped material on the slide 14.

The light calibrating material 45 which is in the form of a cross 52 shown in FIG. 5C may also take a large number of different shapes and forms, and in fact, may be merely the border 54 for the control cell objects or may be a logo CAS or other identifying mark which has been applied to the slide to provide a predetermined optical density when the light source for the microscope 15 is adjusted to the desired intensity.

The present invention is also useful for later analysis of the specimen cells 12 on the slide 14; and to aid in the recall of cell images stored in memory or to allow the operator or another person to return to a given cell for a second review thereof at a later time. To this end, after the slide 14 has been secured on the microscope stage 51 (FIG. 1) in a particular position, a certain location or landmark on the slide, such as the center of the cross 52, is defined as a zero-zero X-Y reference point. This reference point is used to set up a translation table for the position readings from the X, Y sensors. A pair of location registers of the system control 22 for the X and Y distances are zeroed at this point so that subsequently all cell locations may have a specific X and Y coordinate address from the reference point. Another easy landmark to find with the position adjustment means of the microscope stage 51 is a corner such as the righthand lower corner 53 of the box border 54 within which are located the reference cell objects 40. The box border 54 is printed on the slide and it also may be used for the optical density calibration rather than the special cross 45. By suitable logic, any point on the slide and microscope

stage at which the classification operation begins may be taken as the zero X and Y location with the location registers. The X and Y coordinates are initially zeroed at this location and then provide a readout for each image field location from this zero location.

The specimen slide 14 may be of any size or shape but because of the familiarity of laboratory technicians and pathologists with glass slides used with microscopes, it is preferred that the support 14 be an actual microscope slide of glass which typically measures 3 inch by 1 inch. The illustrated slide 14 shown in FIGURE 4 has a preprinted border 54 within which are located reference or control cell objects 40. The slide also has an area 61 for specimen cell objects.

The control cell objects are, in this illustrated embodiment of the invention, lymphoblastoid cells of a known size and shape, and DNA content. The lymphoblastoid cells may be mostly of the type having normal DNA content, although some cells may have double or other ratios to the normal DNA content which is typical of cancer cells. The control cell objects may be other types of cells having dark centers or nuclei which stain well, such as chicken blood cells or trout cells. On the other hand, the cell objects 40 may be artifacts printed on the slide to have a cell shape. Furthermore, as above explained, the cell objects 40 may be conventional plastic beads of a predetermined size which will react with a particular fluorescent stain or enzyme stain when treated simultaneously with specimen cell objects such as monoclonal antibodies used in the specimen area 61 of the slide. The reference cell objects will vary from test to test and the present invention is not limited to any particular test or cell objects.

A pathologist will take a previously prepared slide such as shown in FIGURE 4A having premounted thereon the control cell objects 40 and add thereto the

specimen cell objects 12 which are, in this instance, cells from a slice of tissue (such a tumor tissue), or a a needle aspirate of tumor tissue or monolayer of blood cells or other cells, at the area 61 on the slide. The pathologist will then stain or otherwise treat simultaneously the control cell objects and the specimen cell objects for image enhancement. The preferred slides are provided with a kit which have bottles of reagent therein specific to the control cell objects. For DNA analysis, the kit contains bottles of Feulgen Azure A reagent solution and bottles of rinse reagent solution to specifically and quantitatively stain nuclear DNA.

To prepare a slide, the following process is used. The slide 14 has normal control cells on one area and a space for the specimen cells on another area. The slide 14 is fixed within 10% formalin for ten minutes and then put aside while routine paraffin embedded sections are prepared for the area 61. If a malignant or questionable tissue is present on the permanent section, the slide 14 will be processed to analyze the section.

Processing consists of first treating the slide for 65 minutes in 5N hydrochloride to hydrolyze the nuclear DNA. The slide is then transferred to a container of Azure A stain for a two hour staining period. Afterward, the slide is washed in rinse solution, dehydrated with ethanol, cleared with Zylene, cover slipped, and mounted. At this point, the slide is permanently fixed and ready for analysis at any time.

The system software for DNA analysis can now determine the density of the cellular DNA by obtaining the optical density of the specimen cells via the instrument 11. In general, the mass of a stained cell object can be obtained from its optical density by utilizing Beer-Lambert Law which is well known in the

art of microspectrophotometry. The equation states:

$$M = \frac{\alpha \sum OD}{E\lambda}$$

where M = mass of the object in picograms
α = spot size in μm^2
$E\lambda$ = extinction coefficient of the stain at wavelength λ in $\mu m^2/pg$.
OD = optical density of each spot (dimensionless)

The instrument uses this law to find the mass distribution of a number of cells or cell objects which can then be analyzed according to a statistical basis, histogram, or other analytical format as will be discussed hereinafter. The spot size α is determined by the number of pixels which are measured by the camera 18. The optical density for each pixel is calibrated by adjusting the light level, focus, and reading a reference optical density from the calibration area on the slide. This calibration allows the conversion of the measured light levels for each pixel into an optical density a dimensionless quantity.

A calibration for the extinction coefficient is accomplished by measuring the optical density for a plurality of the control cells to determine a peak for the distribution in relative mass units. Because the peak DNA content is known for the control cell distribution, the cells in the measurement field can be measured using the relative OD units and then converted directly into picograms by using the control cell calibration. For example, if the control cells are known to contain 5.96 pg of DNA (trout cells) and a group of calibration cells show a peak distribution of 11,000 relative OD units then a normal group of human cells (with a known DNA content of 7.18 pg.) would

exhibit a peak in their distribution at approximately 13,250 relative OD units. Further, any other relative OD unit measurement can be converted directly into picograms by determining and using the extinction coefficient found from the group of calibration cells.

The system software for DNA analysis is a menu driven program that uses interactive information screens on monitor 62 to assist the operator in making the described measurements on several cells or cell subpopulations. In FIG. 10 there is illustrated the visual screen structure of the program which appears on monitor 62. A main screen 150 provides information as to the calibration and analysis status of the apparatus and provides an option list for calling three other primary information screens. A pictorial example of the main screen is shown in FIG. 11. The three primary information screens correspond to the three operational functions of the program where the label screen 156 corresponds to the label function, a calibration screen 152 corresponds to the calibration function (optical density and staining), and an analysis screen 154 corresponds to the analysis function. Each screen 152, 154, and 156 provides an option list or menu wherein one of the options is to exit to the main screen 50.

In addition, the operator can enter the calibration screen 152 from the analysis screen 154, or vice versa. Two other screens are available as options from the analysis screen 154 and are used to adjust the boundary of the viewing field with the adjust boundary screen 158 and to display the X, Y coordinates of the fields that have been measured with the X, Y fields screen 160. A pictorial representation of the calibration screen is illustrated in FIG. 12, while pictorial representations of the analysis screen and the X, Y field coordinates screen are illustrated in FIG. 13 and FIG. 14, respectively.

When the unit is in operation the pathologist has a number of options or functions from a menu on each screen from which he can choose to acquire data and process that data. In general, the program is menu driven from the main screen illustrated in FIG. 11 which provides a main menu of options as shown in FIG. 15. The main menu A10 consists of five main screen functions including a label function A12, a calibrate function A14, an analyze function A16, a help function A18, and an exit function A20.

The label function allows a user to enter information regarding patient identification, accession number, and DNA conversion number. The DNA conversion number is the number that the first and second peak masses are divided by to get the first and second peak indexes (FIG. 11). Initially, the number is set to a standard 7.18 picograms/cell for normal human cells. However, the apparatus may be used to measure non-human cells and the index may be changed to that desired. The DNA index number must be greater than or equal to 1.0 and less than or equal to 99.99. If the conversion number is not within that range, the user is not allowed to select the analyze option in either the main or the calibration screens.

The three lines of information entered during the label function will appear on every screen except the X, Y field coordinate screen. The label operation is exited by pressing either the enter or escape key. Pressing the enter key will save any changes that were made to the three lines of information. Pressing the escape key will ignore any changes that were made to the three lines. The information stored during the label function will not be saved when the program is exited.

Selecting the calibrate function will cause a change of the display on monitor 62 from the main screen to the calibration screen illustrated in FIG. 12. The calibration screen whose options are shown in FIG. 16

are those necessary to perform calibration of the instrument for optical density and for staining factor on the control cells. A calibration of the apparatus 11 is to be performed every time a new slide is selected to normalize the light level and staining factor.

Selecting the analyze function will cause a change of the display from the main screen on monitor 62 to the analysis screen as illustrated in FIG. 13. The analysis screen contains the menu for the functions that are necessary to perform data acquisition and DNA measurements on the specimen cells. These functions are more fully set forth in FIG. 17. Three criteria must be met in order to select the analyze function. First, the set light function in the calibration screen must have been successfully performed at least once. The set light function is successful when the current image is blank and the light level is between 129 and 131. Secondly, the calibration control cell count must be between 50 and 512. Finally, the DNA conversion number must be greater than or equal to 1.0 and less than or equal to 99.99.

The exit function allows the user to terminate the operation of the program from the main screen. Pressing the escape key is the same as selecting the exit function. When the exit operation is specified, either by selecting exit or pressing escape, the user will be asked to confirm his command to exit. To accept the confirmation, the user selects the yes key. To reject the confirmation, he selects the no key or presses the escape key.

The options for the calibration menu are illustrated in FIG. 16. The calibration menu choices include a set light function A24, a set X, Y function A26, a focus function A32, a measure function A36, an X, Y function A38, an analyze function A28, a clear function A30, a help function A34, and an exit or return to the main screen function A40.

The set light function A24 calculates the background light level for the current image. The light level can be adjusted until it is within a standardized range. The set light function should be successfully performed at least once before selecting the analyze, focus, and measure functions. For the most accurate results, the light level should be exactly equal to 130. The light level value is displayed on the calibration screen by the words "light level." If the light level is successfully set, image noise subtraction will occur in the measurement program.

The light source calibration achieves a number of results including assistance in focusing the microscope by providing an update of a gray scale histogram which may be in the form generally shown in FIG. 22 entitled "Distribution of Light Intensity". The illustrated histogram shows a comparison of incident light I_o and transmitted light I_t with the transmitted light having grey level value and the incident light having a grey level value. The operator has moved the platform 51 to view the light calibrating cross 52 on the monitor 37. The operator views the light calibration material 45 and the system calculates a histogram of that pattern by inputting light from the picture elements of that field. The operator then adjusts the intensity level of the source 17 to change the light level reading on the screen. The operator does this by alternatively selecting the function and adjusting the source 17 until the correct reading appears on the screen. When the light source has been adjusted to provide the left and right peaks I_t and I_o for the transmitted and incidental light at the desired grey levels, 130 as the background light intensity, the system is calibrated. The system software of the apparatus 11 uses the I_o and I_t values to set up an internal calibration table for optical density, such that light intensity sensed for

each picture element is referred to this table and known to have a particular optical density in the image scene being analyzed.

As is known with digital imaging apparatus the actual optical density for a dark object is known using white (I_o) as a reference. By calibrating the apparatus for optical density, the incoming image data may be converted by the lookup tables in the image processing board 21 so that output optical density can be linearly added to proportionately reflect directly, in this instance, the amounts of DNA in the specimen objects.

The set X, Y of function A26 provides the setting of the origin for the slide X, Y coordinate system. This function sets the current image or field location as the origin by zeroing a pair of location registers in the software. Generally, the microscope platform 51 is moved until a easily recognized landmark is visible, such as cross 52. This landmark is then used to rezero the coordinate system to provide a means of relocating previously measured fields. The set X, Y function is used every time a new slide is selected. If the set X, Y function has not been executed, then the X, Y functions of the calibration and analysis screens and the functions in the X, Y field coordinates screen will not work properly. The set X, Y function can only be used when the calibration control cell count is equal to zero. If the microscope platform 51 is being moved when the set X, Y operation is in execution, then the coordinate origin will be in error. The program provides a message on the screen to notify the operator when the set X, Y operation is successful. In response to the function not being successful, the operator merely reselects set X, Y from the menu and attempts the function again.

The measure function A36 is used to perform the control cell or control object calibration for

normalizing the staining factor. When the measure function is selected, the camera image acquisition will stop and the cursor 170 on the calibration screen will move to the words "measure operations" in FIG. 12. When the cursor 170 is at this location, the user can specify measure operations by activating the numeric lock. An identifier such as a magenta colored box will be placed around an identified cell object. By using a number of key operations illustrated in FIG. 18, the operator can perform an interactive selection and rejection process which will be more fully explained hereinafter.

During control cell calibration, the operator moves the microscope stage by turning the conventional X and Y knobs 11 and 17 (FIG. 1) to shift the control cell objects 40 into view on the monitoring screen 37. When an individual cell object 40 is within a box or indentifier border 75, the operator presses a key on the keyboard 36 to enter measurement of the summed optical density for that control cell object. After a suitable number of control cell objects have been analyzed, the operator will be provided with a histogram such as shown in FIG. 12 on the video monitor 62 which shows the operator the control cell object ploidy distribution as having a relative quantity of DNA. Internally within the system control 22, the summed relative optical density values actually measured for the control cell objects is compared to a predetermined standard or reference amount of DNA which the control cells are known to have. In the present example, if the control cells contain 5.96 picograms of DNA per cell, then an optical density measurement of approximately 8700 relative OD units corresponds to that mass. The actual summed optical density found by the operator is divided into the stored reference DNA value to provide a factor by which to adjust the extinction coefficient for deviations in the stain from a perfect staining.

The X, Y function A38 when selected displays on the calibration screen, the X, Y coordinates of the current image or field, on monitor 37. The coordinates will be continuously displayed until the user presses a key (except CTRL, ALT, or SHFT). Thus, if the same origin for the slide 14 was set, the operator can, by positioning the platform 51 and watching the coordinates change, find the same image which was previously recorded. The set X, Y function A26 must have been successfully performed previously in order for the X, Y function to be selected.

The clear function A30 will cause a purge of all the stored data images. After a clear function has been selected, the user will be asked to confirm the operation. To accept the confirmation, the user selects the yes key to confirm or, to reject the confirmation, the user selects the no key or presses the ESC key.

The focus function A32 provides color enhancement to the image so that the user can perform more precise focusing of the image. The system control 22 automatically provides different colors for gradations in grey level in the image. The operator then adjusts the focusing means of the microscope 15 until the object being focused on, for example an edge of the box, shows a clear color demarkation. This is an indication that the two separate levels or grey scale of the edge are in focus. This is much more difficult without color because the two grey levels may be close together and undiscernible without the color enhancement. The set light function A24 must have been successfully performed at least once in order to select the focus function. To restore the image to its original color, the focus function is selected a second time. If the color enhanced image is present when the user selects the measure function, the image will automatically be returned to its original color.

Selecting the analyze function A28 will change the display from the calibration screen to the analysis screen. The analysis screen provides a menu of functions shown in FIG. 19 that are necessary to perform the DNA measurements on the cellular material. Three criteria must be met in order to select the analyzed function. First, the set light function in the calibration screen must have been successfully performed at least once. Secondly, the calibration control cell count must be between 50 and 512, and finally the DNA conversion number must be greater than or equal to 1.0 and less than or equal to 99.99. The analyze function in the calibration menu works the same way as the analyze function in the main screen.

The analyze function options in the analysis menu are more fully shown in FIG. 19. The analysis menu allows the selection of a classify function 44, a focus function 46, a check light function A48, a select-2nd, function A50, an area 1-2 function A52, a scale function A54, a display X, Y function A56, a boundary function A58, a clear X, Y function A60, a help function A62, a report function A64 and a return to the main menu function A66.

The check light function A48 calculates the light level of the current image. The light level value is displayed on the analysis screen by the words "light level" in FIG. 12.

The select-2nd function A50 allows the user to select the second peak on the histogram displayed on the analysis screen. The mass, DNA index, and the area of the second peak are displayed on the screen under the words "second peak." The select-2nd function cannot be selected when the shown cell count is equal to zero. The shown cell count is displayed by the word "shown." After the select-2nd function has been selected, the cursor will move to a set of arrows and the current second peak location on the histogram will be

highlighted in yellow. Initially the right most histogram data location is chosen as the second peak. Selecting the left arrow moves the second peak location to the left and the user selects the right arrow to move the second peak location to the right. Every time an arrow is selected, the current peak data on the screen will be updated.

Below the histograms horizontal axis, one of three symbols will appear underneath the second peak location. A "less than" symbol will appear if the second peak lies in area one. A "greater than" symbol will appear if the second peak lies in area two. An up arrow symbol will appear if the second peak lies in neither area one nor area two. The reason for the three symbols is so that the second peak location can be identified after the select-2nd operation is exited. The vertical yellow line disappears once the select-2nd function is exited. The users presses the ESC key to exit the select second operation. The second peak data will also be automatically cleared when one of the following analysis screen functions is selected: clear, report, scale, or main.

The classify function A44 allows the user to classify the cells or objects in the current image. After the classify function has been selected, the user will be asked to confirm the operation. To accept the confirmation, the user will select the yes key, and to reject the confirmation, the user will select the no key or press the ESC key. If the classification is confirmed, the camera acquisitional stop and the cursor will move by the words "classify operation." When the cursor is at this location, the user can specify the classify operations. The numeric lock is activated which enables these functions. As was the case in the measure function, a magenta colored box will be placed around a current cell and the operations allow the user to move this cell identifier through the image to identify and classify the cells therein.

The display X, Y function A56 will change the display from the analysis screen to the X, Y field coordinates screen (FIG. 14). The X, Y field coordinate screen will display the X, Y coordinates of the first 512 images that are classified and stored. Also, the screen contains the functions that allow the sorting of the image fields by coordinates. The set X, Y function in the calibration screen must have been successfully performed before the display X, Y function is selected.

The X, Y field coordinate screen has several functions. One of the functions, "nearest" sorts the X, Y coordinates according to the distance from the current X, Y field position. The X function will sort the X, Y coordinates according to the X coordinate value. If there is a tie, then the Y coordinate value will determine the sort order. Similarly the Y function will sort the X, Y coordinates according to the Y coordinate value. If there is a tie, then X coordinate value will determine the sort order. The "field#" function will sort the X, Y coordinates according to the coordinates field number. The field number is the order in which the images were classified.

The page up function allows the user to display the previous page of X, Y coordinates, if any, and the page down function allows the use to display the next page of X, Y coordinates, if any. The exit function changes the display from the X, Y field coordinate screen to the analysis screen. Pressing the escape key is the same as selecting the exit function.

Selecting the X, Y function displays the X, Y coordinates of the current field. The coordinates will be continuously displayed until the user presses a key (except CTRL, ALT, and shift). The set X, Y function in the calibration screen must have successfully been performed before the X, Y function is selected. The X, Y function in the X, Y field coordinate screen works the same way as the X, Y function in the calibration and analysis screens.

The clear function A60 will clear all analysis related areas of data. After the clear function has been selected, the user will be asked to confirm the clear operation. To accept the confirmation, the user selects the yes key, or to reject the confirmation, the user will select the no key or press the ESC key.

The focus function A46 provides color enhancement to the image so that the user can perform more precise focusing of the image. The focus function in the analysis screen works the same way as the focus function in the calibration screen previously described.

The area 1-2 function A52 allows the user to specify two areas in the histogram displayed in the analysis screen. The purpose of this function is to identify the cell counts in certain areas in the histogram. The area 1-2 function cannot be selected when the shown cell count is equal to zero. The cell counts are displayed at the lower right portion of the screen. After area 1-2 is selected, the cursor will move to a row of numbers that is below the histograms horizontal axis. The row of numbers allows the user to specify the locations of area 1 and area 2. The user types a "1" to specify that the current histogram position belongs to area 1. The user types a "2" to specify that the position belongs to area 2. The user types a "0" to specify the current histogram position belongs to neither area 1 or 2. The user is allowed to specify an area 1 without an area 2, but cannot specify an area 2 without an area 1. When both areas are specified, area 1 must be specified to the left of area 2. The area must be specified as continuous. To exit the area 1-2 function, the user presses the enter or ESC keys. If the user presses the enter key, area 1 of the histogram will be highlighted in green and the area 2 will be highlighted in magenta. The area cell counts will also be displayed. Pressing the ESC key will cause the program to disregard any of the changes

that were made. Area 1 and area 2 data will automatically be cleared when one of the following functions is selected: classify, clear, reports, scale or main.

The analysis function A44 is more fully described with respect to FIGS. 20 and 21. The operator will select a number of field locations 360, 361, and 362 in the slide specimen area 61 for analysis. The operator will move the X and Y knobs 11 and 17 for the microscope stage 51 to move into view on the monitor screen 37 a first field of specimen cell objects to be analyzed for DNA content as well as for cell morphology if desired (FIG. 20). The program will place a box, for example at 300, over a particular specimen cell object 12 being displayed on the monitor 37 and then the operator will use a key to cause the scanning of the pixels (picture elements) of the specimen object to classify the cell in a manner similar to that disclosed in U.S. Patent 4,453,266 to give summed optical density for the cell specimen object i.e., a stained cell nucleus, as well as its area, its roundness, and other classification information. Also, the operator has on the keyboard 36 several cell classification keys to be manually operated and the operator depresses one of the keys of a known category such as a type 0 normal cell; a type 1 cancer cell; a type 2 cancer cell; a type 3 cancer cell; and etc. On the monitoring screen 62 there will be an analysis histogram displaying the DNA content of the cells in the field. The operator selects a number of cells in each field or area and then moves the microscope stage to position a number of different fields of specimen cells into view and takes and analyses a number of these specimen cells until the operator feels he has enough cells for a representative sample.

A histogram, such as shown will at this time be displayed on the monitor screen 62 which shows the

number of cells of a particular DNA content and shows the DNA content averages for each of the reference peaks, such as shown in FIG. 13. By depressing a print key, on the keyboard 36 the operator may print out the histogram shown in FIG. 13 on the printer 38. The data for the specimen cells is also stored internally within the system control 22 for later recall and comparison with data of any new specimen from the same patient for analysis relating to the progress or regression of a patient.

The operation of the analysis function will be now more fully described with respect to FIGS. 19 and 20 whereas there is shown a visual field which has been previously stored in the instrument. The field contains a number of cell objects which are to be classified and measured as to DNA content. When the program initially comes into this mode of operation, the first object in the field will be identified by scanning the pixels of the field in a raster like manner until a cell object is recognized. Once a cell object is recognized, an identification means such as box 300 is drawn around the object. This provides a visual identifier for the operator to determine which cell object is presently being measured. In addition to the measurement, the operator has a number of options in the analysis menu. The primary option that an operator has is to classify a current object in block A202. He accomplishes this by pressing one of the numeric keys 0 through 5 which automatically puts the cell object of the identifying box 300 into the classification 0-5 selected. If the object identified is debris, not an abnormal cell, or not an identifiable cell object, the operator can reject the current object by selecting a 9 on the keyboard as indicated in block A204.

After the classification or rejection of the object in box 300, the operator can move the identification box to the next unmeasured object as

determined in block A208. A operator accomplishes this by pressing the keys CTRL/F2 which causes the program to erase the box 300 and search for the next identifiable cell object. This cell object is found and then another identifying box 302 is drawn around it to indicate to the operator the function has been accomplished. In this manner, the entire group of cell objects can be classified and measured or rejected by repeating this process. FIG. 20 illustrates the procedure of scanning for a cell object, putting an identifying box around it, and the classifying or rejecting the object. In this manner, the program steps through the analysis procedure from object 300 to 302, 304, 306, 308, 310, 312, etc.

Further, if one of the cell objects to be classified does not look like the operator thinks it should and cannot be put in one of the previous classifications, or for some other reason the operator believes he has classified a previous object by mistake, then in block A206 by pressing CTRL/F1, he can move the identifying box back to the previously measured object. After identifying all cell objects in the particular field being displayed, the operator has the option of going to another field by manipulating the X, Y positioning mechanism to provide more cells for the particular analysis.

When the operator has determined that enough cell objects had been analyzed, he may either terminate the analysis function by pressing either the enter key or escape key. If he terminates the analysis function by pressing the enter key, as indicated in block A212, then the data assembled for each of the measurements will be saved. However, if the analysis function is terminated by pressing the ESC key, then in block A216 the data will not be saved.

The report function A64 allows the user to specify which cell classifications are to be included in the histogram shown on the screen of the monitor 62.

After the report function has been selected, the cursor can be moved to a option list which will allow the operator to specify the cell types. The following table specifies which key the operator presses in order to select a particular cell type.

CELL TYPE	KEY
normal	0 or n
1	1
2	2
3	3
4	4
lymphocyte	5 or L

Any combination of the types for the report data is allowed. The program will ignore any other characters than those listed in the table. The operator exits the report operation by pressing the enter or escape key. If the operator presses the enter key, he will change the types in the histogram to those which were specified. However, if the escape key is selected, the program will ignore any changes that were made and return normally. The functions area one and area two and the second peak data will automatically be cleared when the report operation is performed.

The scale function A54 allows the operator to change the scale of the horizontal axis of the histogram provided on the analysis screen. There are three scales to choose from, 0-16, 0-32, and 0-64. If the scale function is selected when the current scale is 0-16, then the new scale will be 0-32. If the scale function is selected when the current scale is 0-32, then the new scale will be 0-64. Likewise, if the scale function is selected when the current scale is 0-64, then the new scale will be 0-16. In this function the area 1, area 2, and second peak data will automatically be cleared when the scale operation is performed.

The boundary function A58 will change the display on monitor 27 from the analysis screen to the

adjust boundary screen. The adjust boundary screen contains functions that are necessary to change the cell boundary, i.e., threshold. While addressing the boundary screen, the camera image acquisition will be halted.

The step function allows the operator to change the amount by which the boundary will change when one of the arrow keys is selected. The value must be in the range of 0-128. After the step size is selected, the cursor will move to the location on the screen where the user can type in a new step size value. To exit the step size function, the enter or escape keys are used. Pressing the enter key will save the step size change where pressing the escape key will ignore any change that was made. Initially, the step size value is equal to one.

The up arrow function will increase the cell boundary by the value of the step size and the down area function will decrease the cell boundary by the value of the step size. The exit function changes the display from the address boundary screen to the analysis screen. Pressing the escape key is the same as selecting the exit function.

In general, an interactive data collection and analysis scheme is used by the apparatus for the collection of specific parameters for both the calibration cell objects and the specimen cell objects. Each field which is selected is displayed on the monitor 37 and either the measure operation of the calibration screen or the classify operation of the analysis screen is chosen.

A software flow chart of a subroutine providing the interactive operations for the calibrate key operations and the analysis key operations, FIGS. 18 and 19, is illustrated in FIG. 24. When the operator selects either the measure operations or the classify operations, this program is called to provide the

selection process for both the calibration cell objects and the specimen cell objects. The program begins in block A300 by performing a raster scan of the stored image pixel by pixel until it finds a pixel greater than the threshold value. The test for this operation is performed in block A302. If no pixel is found which is greater than the threshold, block A304 determines if the scan is complete. If not, the program loops back to block A300 where the scan is continued until all pixels in the image field are tested. After all pixels have been tested, the scan parameters are reset in block A308 and the cell object array updated in block A310.

At the time an image pixel is determined to be greater than the threshold, the program will label the object in block A306. The operation of labelling will now be more particularly described. The individualized cell objects in the digitized image are located by a scene analysis technique in which the raster scan is made of the digitized image to locate any pixel above the critical threshold. The technique then performs a four neighbor analysis of adjacent pixel elements and continues in a recursive manner locating "neighbors of the neighbors" which are above the threshold until the entire region of a cell object is defined. This technique is preferred to other scene analysis techniques, such as local boundary found from a gradient image, because it is fool proof in distinguishing the true region of a cell, particularly those cells having irregular or spiculed projections.

The four pixels (top, bottom, right side, and left side) surrounding the initially located pixel which are contiguous therewith are examined sequentially to identify the next pixel with a optical density or gray level value above the threshold. For instance, if the pixel located above the first pixel is not above the threshold, it is discarded from the labelling routine. The next pixel (right side) in a clockwise direction is

then examined and may be above the threshold. If so, that pixel is then identified and stored in memory with the pixel as being a portion of the region of a cell. Next the address and density of the pixel found is stored in a pushdown list and the four neighboring pixels of that pixel are examined in the same clockwise order. This continues in a recursive manner until no neighbors are found above a threshold for a particular pixel. At this point the prior pixels on the pushdown list are reexamined to continue the neighbor search process until the entire number of pixels defining a region, i.e., the cell object has been identified. Thus, each of the pixels above the threshold of the region are identified and a complete enclosed region has been defined for a cell.

Once a cell object is labelled, a cell object table (FIG. 24C) is set up for the object as shown. The table lists the address of its entry point pixel, the number of pixels in the object, the X, Y points for the minimum and maximum points of the object, a count of the pixels in the perimeter of the object, the sum of the optical density of the object pixels, any classification provided for the object, and the X, Y coordinates of the field to which the object belongs. A plurality of the cell object tables comprise a temporary array, called a field array and shown in FIG. 24A, which is used to store the interactive data developed for the present field image under consideration.

In block A312 the program determines whether an automatic flag has previously been set. If so, the program will branch immediately to block A316 or, if not, negatively branch to block A314. Next, in block A313, a box or identifying border is placed around the object using the X, Y limits. This mode identifies a particular object in the field for the operator. In block A314, a key handler is entered to obtain a key press from the operator to determine which of the key

functions of FIGS. 18 or 19 are to be accomplished. The key handler further determines which operation, either for calibration or analysis, is to be performed and only those keys which are associated with the present mode are enabled, all others are locked out. Once a key has been obtained, blocks A326-A342 will determine which function was selected and the progress of the routine.

Keys 0-5 as detected in block A326 provide for the acceptance of a calibration object or the classification of a specimen object. If such key is detected, then an affirmative branch continues the program at block A318. In block A318 the object is relabelled and in block A320 the object is colored (red) to indicate to the operator that it has been accepted or classified. The operator classifies the cell objects into different categories based upon visual clues such as morphology. The cells for analysis can be classified into a normal class 0, or one of several abnormal classes 1-5. The data class of the object is stored to its place in the associated object data table in block A316. Calibration objects are classified as type 0 or normal. The program then returns to the block A300 where the image scan registers are incremented to scan the field for the next object.

Alternatively, if the key press was a 9 as tested for in block A328, this means either a calibration cell object was rejected or a current specimen cell object was rejected. Thus, in block A322 the rejected cell object is colored in a different color (white) than an accepted or classified cell object, and the program returns to the scanning routine entry at block A300 to find another object. Coloring the cell object alerts the operator that the object has been analyzed in this field, coloring the object another color differentiates the object from an accepted or classified cell objects.

If, however, the key press is a CTRL/F1 as tested for in block A336, then the operator desires to move the identifying box to the last previously measured object. The program will then interrogate the field array to find the last object pointer in block A346. This pointer is used to create the box around the previous cell object by transferring control to block A313 before getting another key press in block A314. By using a series of CTRL/F1 keys the operator may selectively move the identifying box from previously measured cell object to previously measured cell object in a reverse direction. If, after the box is placed around a particular cell object, the operator desires to reclassify that cell object, he then has the option of classifying it with the keys 0-5 in block A326.

The identifying box may be moved to the next unmeasured cell object by selecting the key CTRL/F2. The key is tested for in block A338 and if found, immediately returns the program control to the image scan entry in block A300. The effect of this operation is to allow the operator to skip the present cell object and move the identifying box to the next cell object without either rejecting or accepting the present cell. A series of CTRL/F2 presses will move the box forward through the cells without measuring them.

If all of the cell objects in a particular field appear normal as specimen cells, or as is generally the case with control cells they are acceptable, the operator may want to classify them all automatically. To accomplish this, an operator presses the key CTRL/F3. This key press is detected by block A339 and transfers control to block A341 where the automatic mode flag is set. The program then returns to the entry of the image scan in block A300. However, instead of going through the normal sequence of placing a box around the next object and waiting for a key press, the program will loop to automatically classify

the rest of the cells of a field. The automatic mode flag being set is sensed in block A313 and the program automatically transfers control to block A316. Automatically classified cells are categorized as normal or type 0. Thus, a scan and labelling loop will be executed via blocks A300, A302, A306, A313, A316, A318, and A320 until the scan of the entire field has been completed as sensed in block A304.

Another option that the operator can select is the cell cutting function which is entered by pressing the key CTRL/F4. This key is detected in block A342 and transfers control to the cell cutting function operation in block A352. When the CTRL and F4 keys are pressed, the user enters the cell cutting mode. While in this mode, the user is permitted to make cut lines inside the indentifier box. The operator cannot make a cut line over a pixel that belongs to a measured or a rejected cell. A measured cell is a cell that has been classified as type 0, 1, 2, 3, 4, or 5. Numeric lock must be activated in order to perform a cell cutting operation. A cross hair is located where the cut is to take place. The following table lists the cell cutting operations that can be performed plus the key that must be pressed in order to select the desired operation. The function allows the splitting of overlapping cells by artificially making a perimeter between two areas, a cut. Thus, the labelling routine will only label one area as a cell object.

(KEY)	(ACTION)
0	Turn splitting on and off
1	Go down and left one step
2	Go down one step
3	Go down and right one step
4	Go left one step
5	Go to the center of the box
6	Go right one step
7	Go up and left one step
8	Go up one step
9	Go up and right one step
ENTER	Re-do last step (up to 100 pixels)
ESC	Exit cell splitting mode

A step is three pixels. When beginning a new cut, the first pixel will not be cut. For operation 5, the cross hair will not move if the center pixel belongs to a measured or rejected cell.

After the cell cutting is performed in block A352, the scanner registers are set to the entry point of the particular object cut. The program then returns to the scan entry in block A300. Because the cell object has the same entry point but a different perimeter, the labelling routine (block A306) will label the cell object as now cut.

Another option that the operator has is the ability to select any object within a field. The selection of this mode is accomplished by pressing the CTRL/F5 key which is sensed in block A340. An affirmative branch from block A340 transfers control to block A348 where the select object mode is entered.

When the CTRL and F5 keys are pressed, the user enters the selection mode. Numeric lock must be activated in order to perform a selection operation. A cross hair will appear at the current selection point. The following table lists the selection operations that can be performed plus the key that must be pressed in order to select the desired operation.

(KEY)	(ACTION)
0	Select cross hair movement step size [5 or 15]
1	Go down and left one step
2	Go down one step
3	Go down and right one step
4	Go left one step
5	Go to the center of the image
6	Go right one step
7	Go up and left one step
8	Go up one step
9	Go up and right one step
ESC	Exit selection mode

When the selection mode is exited, the box will move to the first unmeasured cell after the selection

point. If there are no cells after the cross hair, the box will go to the next unclassified cell.

After the object is selected by the above described technique, the scanner registers are set to the entry point of that particular object in block A348 and the program returns to the scan entry in block A300. This creates the identifier box around the object using its X, Y limits and provides the operator with the option of then pressing another key and performing other measurements and classifications on that selected object.

Another function is provided by key CTRL/F6 which is tested for in block A330. This feature provides an operator with the ability to move the indentifier box forward by reading the next cell object pointed in block A324 and then drawing around the box the chosen object in block A313. The key CTRL/F1, CTRL/F2 thereby allows an operator to quickly revise pervious cell classification by stepping forward and backwards, respectively, through the pointers of the previously measured cells.

When the enter key is sensed, in block A332, control of the program is transferred to block A310. In that block, the cell object array (FIG. 24B) is updated with the present field array to store all of the data collected for the particular objects in the field. Alternatively, the sensing of the escape key returns the program immediately to the place in the software where it was called.

It will be appreciated that the illustrated control 22 has been programmed to do the cell classification and optical density analysis. Such classification and analysis is similar to that outlined in U.S. patent 4,453,266 for the classification or red blood cells and the present invention can be particularly useful in the analysis of red blood cells wherein the optical density of the hemoglobin content is

measured rather than the DNA content as above described. As common in red blood cell analysis, the red blood cells need not be stained for image enhancement so that the staining calibration step may be eliminated for red blood cells when using the specific wave length of light specified in the aforementioned Bacus patents.

A further use of the present invention is to provide a precise measurement of hemoglobin in actual picograms for calibrating other instruments such as a Coulter counter. In such a process, the control blood cells 40 will have a known predetermined hemoglobin value and the specimen blood cells 12 of unknown hemoglobin value will be placed on the specimen area 61. Then the apparatus will be calibrated to show the histogram for the hemoglobin content of the specimen cells 12.

It will also be appreciated that the various calibration steps may be eliminated or combined and done simultaneously rather than done in the order and in the sequence and in the manner described for the preferred embodiment of the invention in making a DNA analysis. The use of the present invention for antigen analysis may include the steps of binding of monoclonal antibodies to the specimen and control cell objects. Later the monoclonal antibodies may be conjugated with an enzyme stain. Also, the monoclonal antibodies may be conjugated with a fluorescent material. Thereafter, the fluorescent stain may be excited at a wave length which excites the fluorescence and the specimen objects observed at another wave length at which fluorescent emission occurs. When the antigen is made for a particular virus, the control cell specimen objects may be treated with a nucleic acid probe specific for the genome of the virus.

While a preferred embodiment of the invention has been illustrated, it will be obvious to those

skilled in the art that various modifications and changes may be made thereto without departing from the spirit and scope of the invention as defined in the appended claims.

WHAT IS CLAIMED IS:

1. A method of analyzing cell objects on a support in automatic analyzing apparatus comprising the steps of:

providing a support means having control calibration material and specimen cell objects in the automatic cell object analyzing apparatus,

calibrating the automatic cell object analyzing apparatus by analyzing calibration material on the support means with light and imaging apparatus and adjusting the apparatus based on the analysis, and

measuring and analyzing the specimen cell objects on the support means.

2. A method in accordance with Claim 1 in which the calibration material comprises the control cell objects and the method includes, prior to calibration, the step of treating the control cell objects and the specimen cell objects at the same time with an image enhancing material.

3. A method in accordance with Claim 1 in which the calibration material is an optical density reference material and in which the calibrating step includes adjusting the optics of the apparatus relative to a reference material of known optical density.

4. A method in accordance with Claim 3 including the step of using the calibration material also as a reference location mark to simultaneously provide a reference location for the support means at the time of adjusting the optics.

5. A method in accordance with Claim 1 in which the calibration material comprises control cell objects and in which specimen cell objects are cells and including, prior to calibration, the step of staining the control cell objects and specimen cell objects at the same time.

6. A method in accordance with Claim 5 in which the staining step comprises staining DNA in the cells.

7. A method in accordance with Claim 6 including the step of staining DNA within the nucleus of specimen and control cells.

8. A method in accordance with Claim 1 in which the reference material comprises control cell objects and including the step of binding the same monoclonal antibodies to the specimen and control cell objects.

9. A method in accordance with Claim 8 including the further step treating the control and specimen cells objects with an image enhancing material.

10. A method in accordance with Claim 9 in which the monoclonal antibody is conjugated with an enzyme and wherein the treating step comprises applying thereto a substance with which the enzyme reacts to provide image enhancement.

11. A method in accordance with Claim 10 wherein the monoclonal antibody binds to estrogen receptors in the specimen and control cell objects.

12. A method in accordance with Claim 8 in which the monoclonal antibody is conjugated with a florescent material.

13. A method in accordance with Claim 12 including the step of exciting the florescent material on the cell objects at a wave length which excites the florescence of said material and analyzing by the step of observing at another wave length at which florescent emission occurs.

14. A method in accordance with Claim 1 in which the analysis is for a particular virus and in which the control and specimen cell objects are treated with a nucleic acid probe specific for the genome of said virus.

15. A cell analysis method for determining the optical density of specimen cell objects, said method comprising the steps of:

providing a support with a reference area and a specimen area;

providing reference cell objects of a predetermined optical density in said reference area;

providing specimen cell objects of an unknown optical density in said specimen area;

measuring the optical density of the reference cell objects

determining a density factor from said measured optical density of said reference cell objects and said predetermined optical density of said reference cell objects;

measuring the optical density of said specimen cell objects; and

determining the true optical density of said specimen cell objects from said measured optical density of said specimen cell objects and said density factor.

16. A cell analysis method as set forth in Claim 5 further comprising the step of:

determining a physical characteristic of said specimen cell objects from said determined true optical density.

17. A cell analysis method as set forth in Claim 6 wherein:

said reference cell objects and specimen cell objects are red blood cell objects and said determined physical characteristic is mean cell hemoglobin.

18. An apparatus for automatically analyzing cells of a specimen as set forth in Claim 5 wherein:

said reference parameter is a factor indicating the change in optical density of the reference cell objects due to the staining process.

19. An apparatus for automatically analyzing cells of a specimen as set forth in Claim 8 wherein:

said stain preferentially stains portions of the reference cell objects and the specimen cell objects.

20. An apparatus for automatically analyzing cell objects of a specimen as set forth in Claim 5 wherein said means for calibrating includes:

means for displaying the distribution of light intensity from the calibration area of the slide; and

means for adjusting the light source in a direction such that the displayed distribution substantially matches a reference distribution.

21. An apparatus for automatically analyzing cells of a specimen as set forth in Claim 20 where said calibration area is divided into pixels each having a light intensity associated therewith, and said means for displaying includes:

means for displaying a range of different light intensities and the number of pixels of said calibration area which have said different intensities.

22. A microscopic slide for an automated cell analysis system said slide comprising:

a support having specimen cell object area and reference area on one side of said support, said reference area containing reference means having predetermined physical characteristics which can be detected by the apparatus for calibrating the apparatus.

23. A microscopic slide as set forth in Claim 22 wherein said support further includes:

an identification area having a prefixed optical pattern which can be identified by said apparatus for verifying the integrity of the slide.

24. A microscopic slide as set forth in Claim 22 wherein said reference area further includes:

a focusing area having an optical pattern of a predetermined optical density which can be measured and identified by said apparatus.

25. A microscopic slide as set forth in Claim 33 wherein:

said optical identification pattern can be identified by measuring the physical dimensions between one or more identifiable features of said pattern.

26. A microscopic slide as set forth in Claim 22 wherein:

said reference area is delineated by a pattern which can be detected visually.

27. A microscopic slide as set forth in Claim 22 wherein said slide further includes:

an identifiable feature which acts as a location reference for the automated cell analysis system.

28. A method of analyzing cell objects on a support usable with an automatic cell analyzing apparatus,

placing specimen cell objects on the support,

putting the support in the apparatus for analyzing the cell objects ,

performing an integrity check on the support to determine if it is a valid support for use in the cell analyzing apparatus,

disabling the apparatus from analysis of the specimen cell objects if the support is not validated,

and enabling analysis of the cell objects on the support if the support is validated.

29. A method in accordance with Claim 28 in which the step of performing the integrity check comprises optical examining the support for a predetermined optical reference.

30. An automated cell analysis system comprising:

a microscope with focusing means;

a specimen slide with specimen cells for mounting on said microscope and having at least one physical characteristic distinguishing whether said slide is authorized for use in the cell analysis system;

a light source to illuminate specimen cells on said slide when mounted on said microscope;

positioning means for positioning said microscope and said slide relative to one another;

means for converting the optical density of a particular location of said slide into an optical value;

means for receiving said optical values and for analyzing characteristics of said specimen cells therefrom;

means for receiving said optical values and for identifying said at least one physical characteristic of said slide; and

means for enabling said specimen analyzing means if said at least one physical characteristic of said slide is identified, and for disabling said specimen analyzing means otherwise.

31. An automated cell analysis system as set forth in Claim 30 wherein:

said at least one physical characteristic is an optical pattern identified by analysis of said optical values.

32. An automated cell analysis system as set forth in Claim 31 wherein:

said optical pattern is identified by its physical dimensions.

33. An automated cell analysis system as set forth in Claim 31 wherein:

said optical pattern is identified by its average optical density.

34. An automated cell analysis system as set forth in Claim 31 wherein:

said optical pattern is identified by having a plurality of identifiable features.

35. An automated cell analysis system as set forth in Claim 34 wherein:

said optical pattern is identified by the physical dimensions of said identifiable features.

36. An automated cell analysis system as set forth in Claim 34 wherein:

said optical pattern is identified by the optical density of said identifiable features.

37. An automated cell analysis system as set forth in Claim 34 wherein:

said optical pattern is identified by the physical dimensions separating said identifiable features.

38. An automated cell analysis system as set forth in Claim 30 wherein:

said at least one physical characteristic is not identifiable without aid of said microscope.

39. An automated cell analysis system as set forth in Claim 30 wherein:

said at least one physical characteristic is not identifiable without aid of said identifying means.

40. A cell analysis method for determining the optical density of specimen cells, said method comprising the steps of:

providing a slide with a reference area and a specimen area;

providing reference cells of a predetermined optical density in said reference area;

providing specimen cells of an unknown optical density in said specimen area;

staining both said reference cells and said specimen cells with the same stain;

measuring the optical density of the stained reference cells;

determining a staining factor from said measured optical density of said stained reference cells and said predetermined optical density of said reference cells;

measuring the optical density of said stained specimen cells; and

determining the optical density of said specimen cells from said measured optical density of said stained specimen cells and said staining factor.

41. A cell analysis method as set forth in Claim 40 wherein said step of staining includes the step of:

preferentially staining only certain portions of said reference cells and said specimen cells.

42. A cell analysis method as set forth in Claim 41 wherein said step of preferentially staining said reference cells and said specimen cells includes the step of:

preferentially staining the nuclei or contents of the nucleus of said reference cells and said specimen cells.

43. A cell analysis method as set forth in Claim 42 wherein:

said specimen cells are nucleated.

44. An apparatus for automatically analyzing cells of a specimen slide mounted on a microscope illuminated with a light source, said apparatus comprising:

means for calibrating the light source on the basis of the distribution of light intensity from a calibration area on the slide;

means for analyzing the cells of a specimen on the basis of the distribution of light intensity from a specimen area on the slide; and

means for enabling said analyzing means on the basis of the distribution of light intensity from an identification area on the slide.

45. An apparatus for automatically analyzing cells of a specimen as set forth in Claim 44 wherein the slide includes a reference area containing reference cells which further includes:

means for calculating a reference parameter on the basis of the light intensity from the reference area on the slide.

46. An apparatus for automatically analyzing cells of a specimen as set forth in Claim 45 wherein:

said reference parameter is used to calibrate said means for analyzing the cells of a specimen.

47. An apparatus for automatically analyzing cells of a specimen as set forth in Claim 46 wherein:

said reference cells and said specimen cells are stained with the same stain.

48. An apparatus for automatically analyzing cells of a specimen as set forth in Claim 42 wherein:

said reference parameter is a factor indicating the change in optical density of the reference cells due to the staining process.

49. An apparatus for automatically analyzing cells of a specimen as set forth in Claim 48 wherein:

said stain preferentially stains portions of the reference cells and the specimen cells.

50. An apparatus for automatically analyzing cells of a specimen as set forth in Claim 44 wherein said means for calibrating includes:

means for displaying the distribution of light intensity from the calibration area of the slide; and

means for adjusting the light source in a direction such that the displayed distribution substantially matches a reference distribution.

51. An apparatus for automatically analyzing cells of a specimen as set forth in Claim 50 where said calibration area is divided into pixels each having a light intensity associated therewith, and said means for displaying includes:

means for displaying a range of different light intensities and the number of pixels of said calibration area which have said different intensities.

52. An apparatus for automatically analyzing cells of a specimen as set forth in Claim 43 wherein:

said reference distribution is indicative of the number of pixels of said calibration area which should exhibit a particular intensity.

53. An apparatus for automatically analyzing cells of a specimen as set forth in Claim 44 wherein said means for analyzing includes:

means for determining the quantity of DNA contained in a specimen cell.

54. An apparatus for automatically analyzing cells of a specimen as set forth in Claim 53 wherein said means for analyzing further includes:

means for determining the number of cells of the specimen having a relative DNA content.

55. An apparatus for automatically analyzing cells of a specimen as set forth in Claim 54 wherein said means for analyzing further includes:

means for displaying the distribution of cells of the specimen to indicate the number of cells having a relative DNA content over a range of DNA content values.

56. An apparatus for automatically analyzing cells of a specimen as set forth in Claim 44 further including:

means for moving said slide so that different areas on the slide can be analyzed; and

means for determining the location on the slide being analyzed:

57. An apparatus for automatically analyzing cells of a specimen as set forth in Claim 56 wherein said means for determining includes:

means for determining a reference location on said slide in accordance with the light distribution of a location area on said slide.

58. A method of measuring the hemoglobin in specimen blood cells by an optical density measuring apparatus, said method comprising the steps of:

providing a slide with control blood cells having a predetermined hemoglobin value,

placing on the slide the specimen blood cells,

calibrating the optical density apparatus to the known hemoglobin value of the control cells.

measuring the optical density of the specimen cells, and

providing an output indicative of the hemoglobin value for the specimen blood cells.

59. A method in accordance with Claim 58 including the step of the measuring the hemoglobin value of each of the individual specimen cells, and calculating a mean cell hemoglobin for the specimen cells.

60. A microscope stage locating apparatus comprising

a microscope stage moveable in the X and Y directions,

a X direction sensor for the microscope stage including a strip having a indicia scale thereon and including a sensing head for sensing the scale with relative movement between the strip and reader head in the X direction,

A Y direction sensor for the microscope stage including a strip having a indicia scale thereon and including a sensing head for sensing the scale with relative movement between the strip and reader head in the Y direction,

electrical output means connected to each of the heads and transmitting therefrom digital output signals representative of the X and Y locations of the microscope stage.

61. An interactive method of analyzing cell objects for a given parameter, said apparatus comprising the steps of:

preparing specimen cell objects for analysis and locating the specimen cell objects adjacent control cell objects,

treating the specimen and control cell objects in a similar manner to assist in the image analysis thereof,

viewing enlarged images of the specimen cell objects in each of a plurality of successive fields,

selecting certain images of specimen cell objects in the respective fields for image analysis and rejecting other images in the fields for image analysis, and

analyzing the selected cell objects for quantitative data for the given parameter.

62. A method in accordance with Claim 60 including the step of storing the location of each field for use in relocating the field for a subsequent review of the cell objects in the relocated field.

63. A method in accordance with Claim 61 including the further step of

manually classifying the selected cell objects into broad classes.

64. A method in accordance with Claim 63 including the further step of classifying the selected cell objects into classes of normal cells and different types of cancer cells.

65. An interactive method of analyzing a subpopulation of cell objects for a given parameter, said method comprising the steps of:

providing an enlarged view of cell objects to the user-observer through digital image processing,

gating on user-observer's viewed morphological criteria each of several cell objects into one of several gated subpopulations of cell objects, and

generating a parameter distribution of at least one of the gated subpopulations.

66. A method in accordance with claim 65 in which the step of gating includes the step of gating the malignant cell objects into at least one subpopulation and in which the generating step includes the measurement of DNA in the gated malignant cell object subpopulation.

67. A method in accordance with claim 65 in which the gating step includes the user gating the cell objects into a normal cell object subpopulation and into one of several abnormal cell object subpopulations.

68. A method in accordance with claim 65 in which said subpopulation of the cell objects is a small fraction of the total population of cell objects.

69. A method of analyzing cell objects to provide a high degree of precision for a display of a parameter distribution of cell objects, said method comprising the steps of:

examining cell objects by image analysis and measuring the cell objects for a given parameter,

storing measurements of the cell parameter into storage bins according to a size of measurement at a first fine resolution,

consolidating the stored parameter information from the storage bins into a coarse resolution for display,

displaying the coarse resolution of the consolidated parameter information as a parameter distribution, and

reporting a fine resolution quantitative distribution parameter for the displayed consolidated and coarse resolution parameter information.

70. A method in accordance with claim 69 including the step of generating a mode of the DNA content of the cell objects and displaying the mode as said fine resolution quantitative parameter along with the display of coarse resolution of the DNA content of the cell objects.

71. A method in accordance with claim 69 including the step of gating the cell objects on user-observer's morphological criteria into subpopulations and generating the mode of the DNA content of the cells of the subpopulation.

72. An interactive method of analyzing cell objects on a carrier by image analysis comprising the steps of:

providing specimen cell objects and calibration cell objects on a carrier,

calibrating the image analysis apparatus by examining the calibration cell objects and measuring a cell object parameter and relating this measurement to an actual mass number,

analyzing the specimen cell objects by image analysis and measuring the parameter as related to a mass number obtained during the calibration process,

generating a parameter distribution of the measured parameter for a population of specimen cell objects, and

reporting the parameter distribution on a coordinate scale according to mass units.

73. A method in accordance with claim 72 which the actual mass number is the DNA in cells in picograms.

74. A method in accordance with claim 73 including the step of reporting a DNA index number.

75. A method in accordance with claim 72 including the step of reporting a main peak for the parameter distribution and reporting a second peak for the parameter distribution.

76. A method of analyzing cell objects on a carrier by image analysis comprising the steps of:

examining cell objects by image analysis for at least one cell parameter, measured storing the cell parameters according to DNA mass,

generating a parameter distribution of the measured parameter showing a first peak and a second peak with a calculated parameter distribution number for the first peak,

observing the location of and selecting the second peak and operating the apparatus to calculate a parameter distribution number for the second peak.

77. A method in accordance with claim 76 including the step of generating the mode for the first peak and the second peak.

78. A method for validating the classification of cell objects on a slide using an image analysis system having a means for generating the position of a movable platform, means for mounting the slide on the platform, means for viewing an image area of the slide,

a memory for storing data, and means for displaying the position data from the means for generating the position of the platform; said method comprising the steps of:

locating the slide at a reference location on the platform;

moving the platform to view a landmark on the slide with said viewing means;

storing the position of said landmark;

translating the position data from said platform position data generating means into slide position data based upon the reference slide location and said landmark location;

storing the slide position data for a particular image area corresponding to a visual classification of said cell objects in that area;

displaying the translated slide position data on said display means;

whereby the image area corresponding to a stored slide position can be positioned for viewing to validate a previous classification by moving the platform until the displayed location of the image area is equivalent to the stored location of the image area.

79. A method as set forth in Claim 78 which further includes the steps of:

reading a first linear sensor for determining one coordinate of the location of the slide; and

reading a second linear sensor located on the platform orthogonal to said first sensor for determining another coordinate of the location of the slide.

80. A method as set forth in Claim 78 which further includes the steps of:

reading said first and second linear sensors on a periodic basis; and

displaying said periodic reading such that a real time position for the slide is displayed on said display means.

81. A method as set forth in Claim 78 wherein said step of storing the slide position data further includes the step of:

storing the visual image corresponding to said slide position data.

82. A method as set forth in Claim 78 wherein the cell objects are cells from a human containing DNA and the step of preparing includes the step of:

selectively staining the cell objects with Azure A Fulegen stain to enhance the contrast of the DNA compared to the other features of the cells.

83. A method as set forth in claim 78 wherein the step of measuring a parameter includes:

quantifying the mass of DNA in said stained cell objects by measuring the optical density of the stained cell objects.

84. An image analysis apparatus for analyzing and classifying cell objects and for validating the classification of cell objects on a slide at a later time by a manual viewing of the previously classified cell objects, said apparatus comprising; an image analysis means having a microscope for viewing the cell objects and including a movable platform for supporting a slide having cell objects thereon,

means on said platform for mounting the slide at a predetermined location on the platform,

means to generate a landmark location on the slide and for generating a location position for each of a number of viewing fields to be analyzed,

image analysis means for viewing cell objects on the slide and for classifying the cell objects into classes,

means for storing the classifications of the respective cell objects in the memory and for storing the location positions of the cell objects on the slide for a later retrieval; and, viewing means for manual viewing of the cell objects previously classified to

allow the operator to later validate the original classification of the cell objects by viewing selectively cell objects in particular classes.

85. An image analysis apparatus according to claim 84 including manually operated means for manual classifying the cells by type such as normal or cancerous at the time of original classification by said image analysis means.

86. An image analysis apparatus in accordance with claim 84 including a slide having a landmark thereon for establishing zero x and y coordinates from which the other location positions for the cell objects on the slide are determined.

87. An apparatus in accordance with claim 84 including means for displaying the location positions for the platform on the viewing means so that the operator may move the platform to the stored location position and the cell object simultaneously.

88. An apparatus in accordance with claim 84 in which said image analysis means shows a field on said viewing means having a plurality of cell objects in the field and in which said viewing means shows a location of the field containing several objects thereby allowing the operator to return to the field and to find and locate several cell specimens or object in said field.

89. A method of image analysis for analyzing and classifying cell objects and for validating the classification of cell objects on a slide at a later time by a manual viewing of the previously classified cell objects, said method comprising the steps of:

providing an image anlaysis means having a microscope for viewing the cell objects and including a movable platform for supporting a slide having cell objects thereon,

mounting the slide on the platform at a predetermined location on the platform,

generating and storing a landmark location on the slide and generating and storing location positions for each of a number of viewing cells to be analyzed,

analyzing the cell objects on the slide by image analysis techniques and classifying the cell objects into classes,

storing the classification of the respective cell objects and storing the location positions of the cell objects on the slide for a later retrieval; and,

reinserting the slide at a later time onto said platform and locating said landmark position on said slide and returning to one of the cell obvjects at its location position and viewing the cell object for validation of its earlier classification.

90. A method in accordance with claim 89 including the step of the operator classifying the cells by type such as normal or cancerous at the time of original and automated classification by said image analysis means.

91. An image analysis apparatus in accordance with claim 90 including the step of moving the platform and the previously classified slide with the landmark thereon and reviewing the zero x and y coordinates from the landmark position until the x and y coordinates for cell object being checked is reached.

92. A method in accordance with claim 89 including the step of displaying the location positions for the cell objects on the viewing means so that the operator may move the platform to a previously stored location position and viewing the location position and the cell object simultaneously.

93. A method in accordance with claim 89 including the step of showing a field on said viewing means having a plurality of cell objects in the field and showing the location of the field containing several objects, and rejecting certain cell objects and selecting other cell objects in said field for original classification.

94. A method validating a patient's cell analysis from a slide having a patient's cell objects thereon, comprising the steps of:

locating the slide at a predetermined position and establishing a zero position on the slide from which locations of the cell objects are measured;

performing an image analysis of cell objects of a patient and classifying the patient's cell objects,

storing identification of the patient and storing locations of the classified cell objects on the slide,

providing a report on the classification of the patient's cell objects, and

reviewing the classifications of the patient's cell objects at a later time by relocating the slide at said predetermined position and then moving the slide to view cell objects at a previously stored location.

FIG. 1

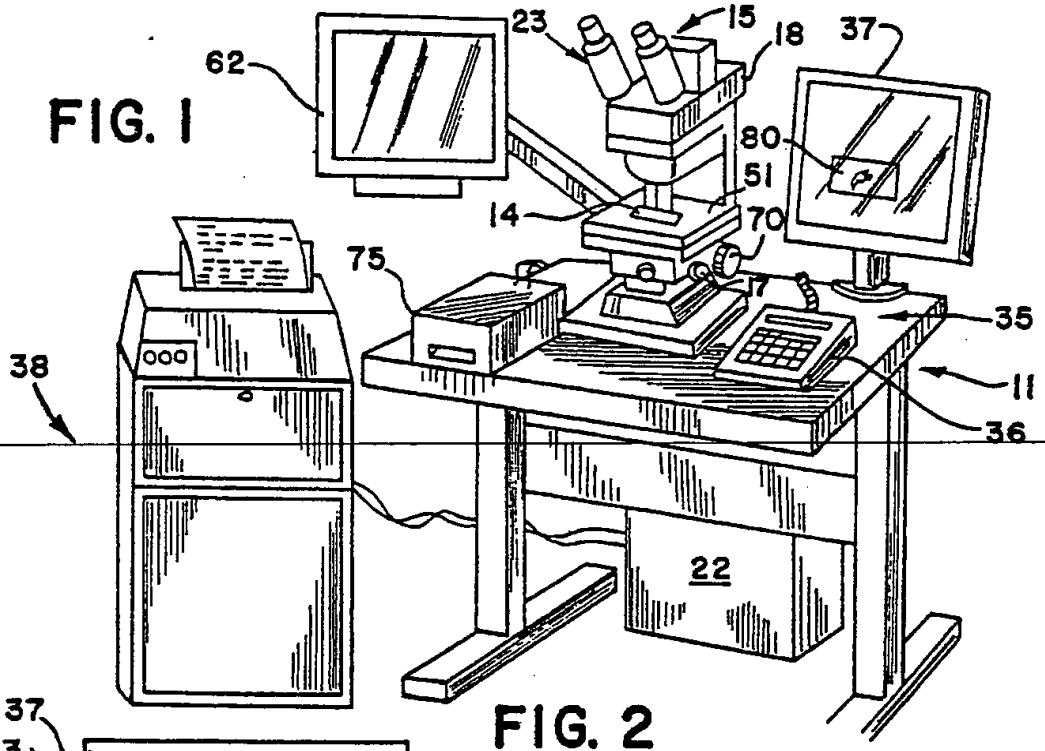

62
23
15
18
37
80
51
14
70
75
7
35
38
11
36
22

FIG. 2

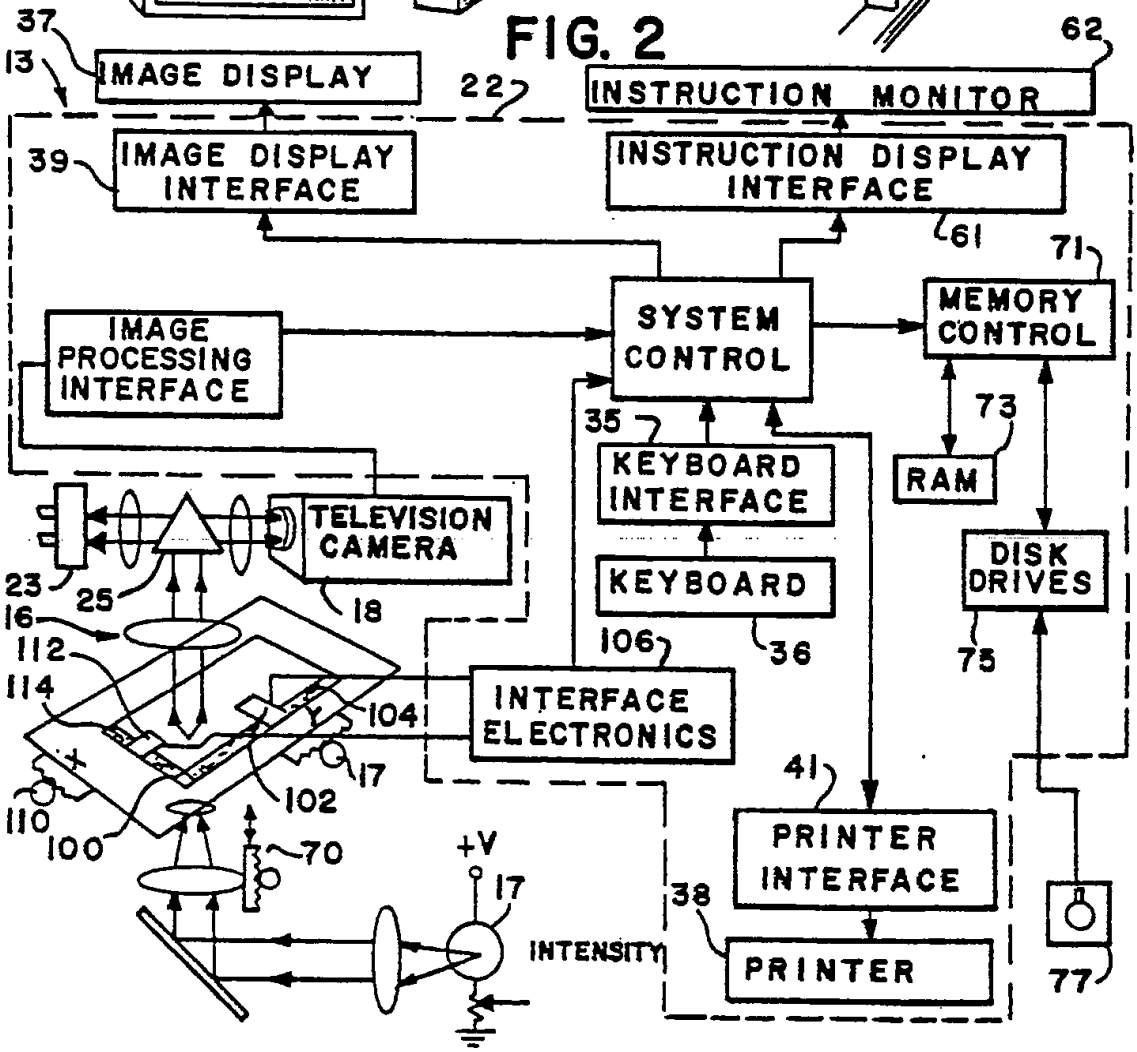

37
13
IMAGE DISPLAY
22
INSTRUCTION MONITOR
62
39
IMAGE DISPLAY INTERFACE
INSTRUCTION DISPLAY INTERFACE
61
71
IMAGE PROCESSING INTERFACE
SYSTEM CONTROL
MEMORY CONTROL
73
35
KEYBOARD INTERFACE
RAM
TELEVISION CAMERA
KEYBOARD
DISK DRIVES
23
25
18
16
112
106
36
75
114
INTERFACE ELECTRONICS
104
17
41
102
110
100
70
+V
17
PRINTER INTERFACE
38
INTENSITY
PRINTER
77

FIG. 2A

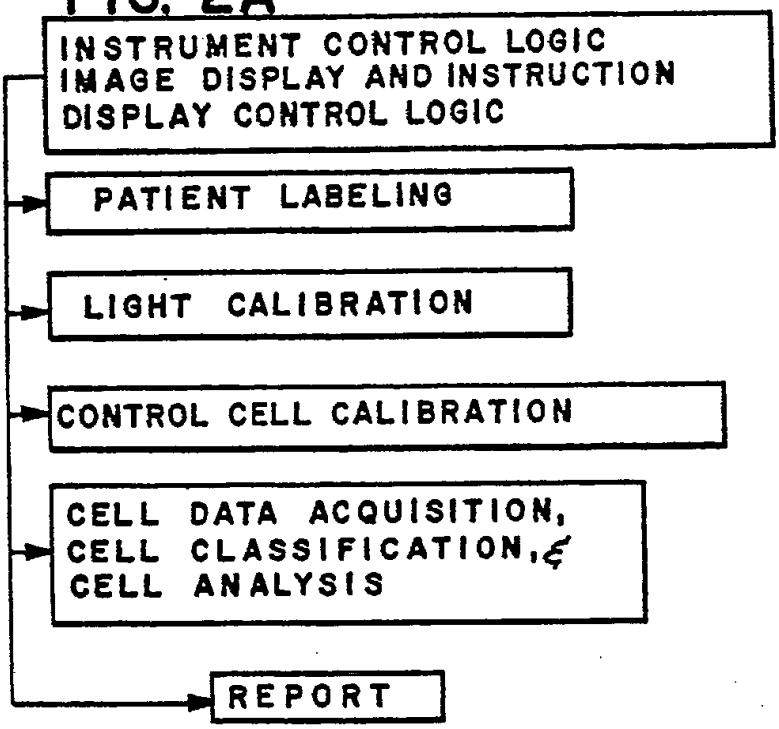

INSTRUMENT CONTROL LOGIC
IMAGE DISPLAY AND INSTRUCTION
DISPLAY CONTROL LOGIC
PATIENT LABELING
LIGHT CALIBRATION
CONTROL CELL CALIBRATION
CELL DATA ACQUISITION,
CELL CLASSIFICATION, &
CELL ANALYSIS
REPORT

FIG. 3

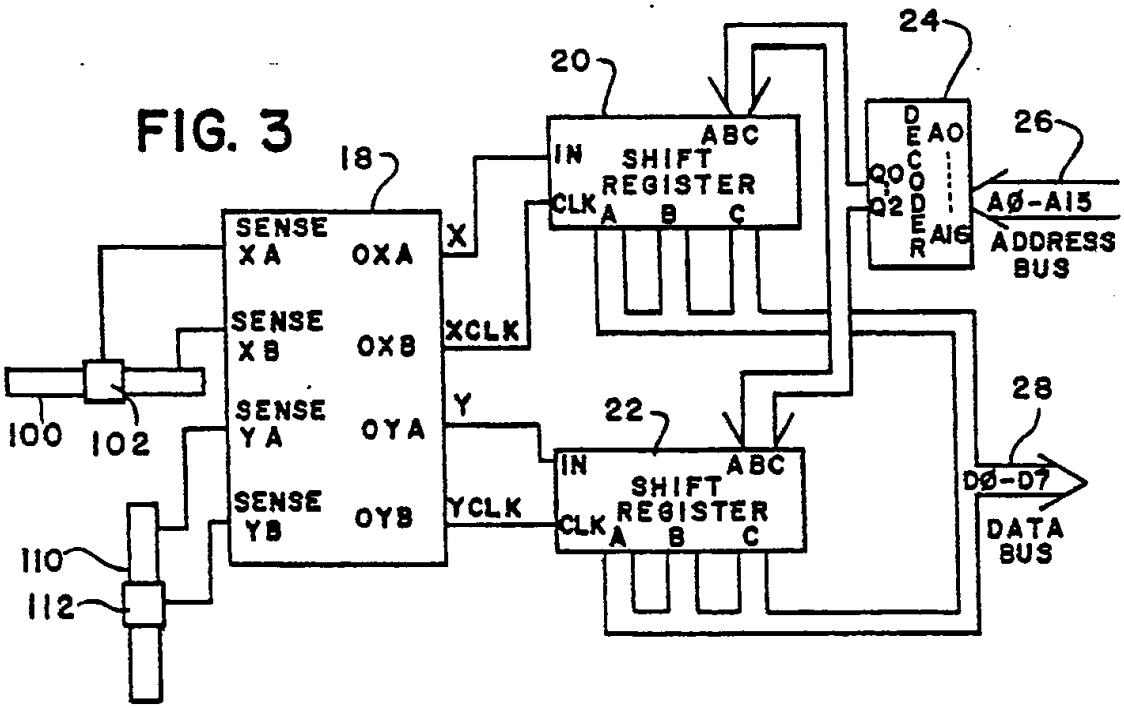

18
20
22
24
26
28
SENSE XA
OXA
SENSE XB
OXB
SENSE YA
OYA
SENSE YB
OYB
X
XCLK
Y
YCLK
IN
SHIFT REGISTER
CLK
A
B
C
ABC
DECODER
A0
A15
Q0
Q2
A0-A15
ADDRESS BUS
D0-D7
DATA BUS
100
102
110
112

FIG. 4

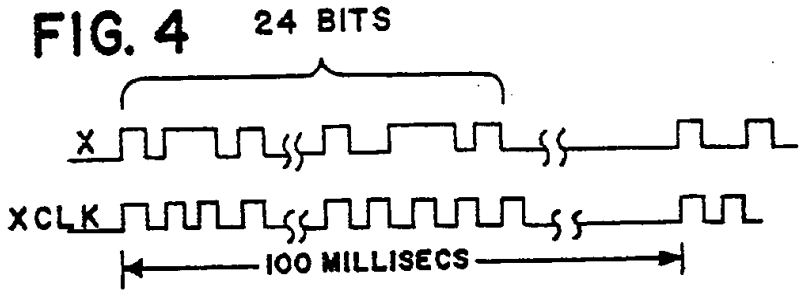


FIG. 5A

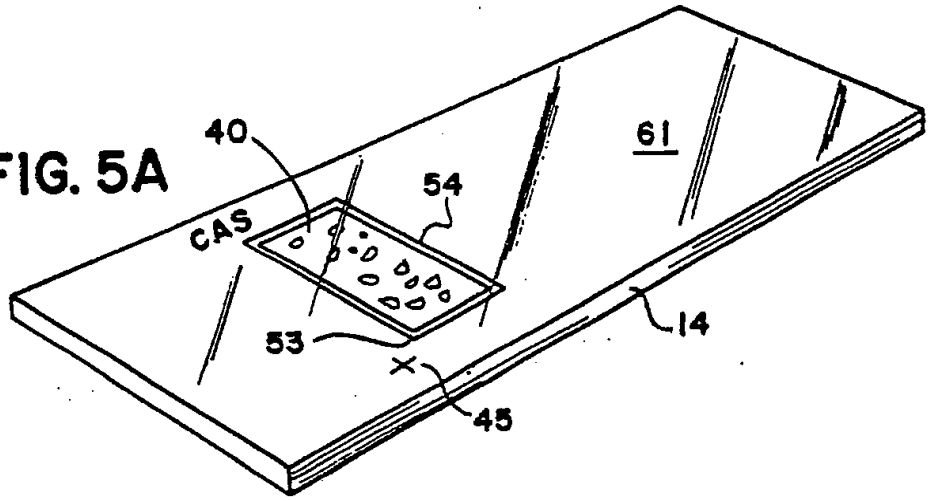


FIG. 5B

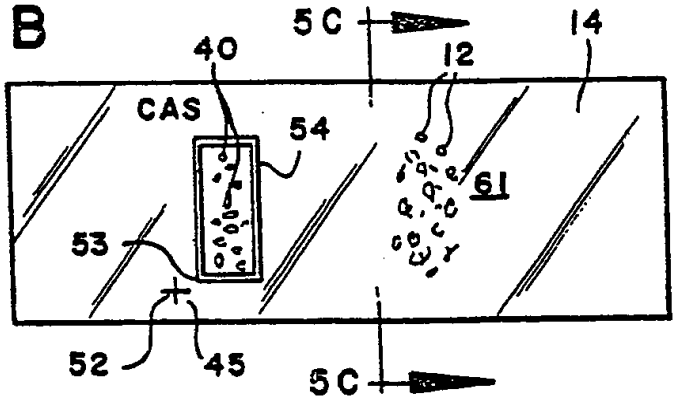


FIG. 5C

FIG. 6

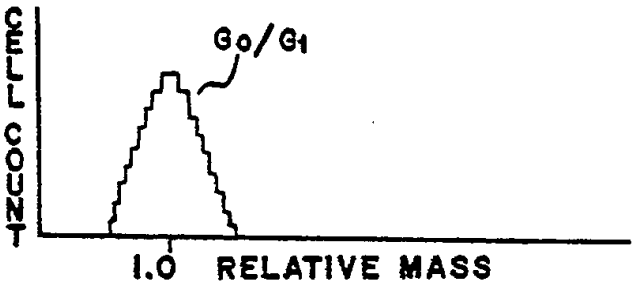


FIG. 7

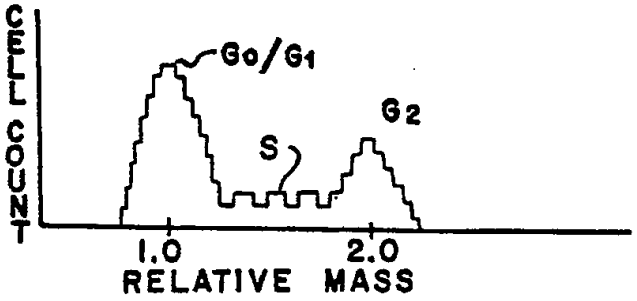


FIG. 8

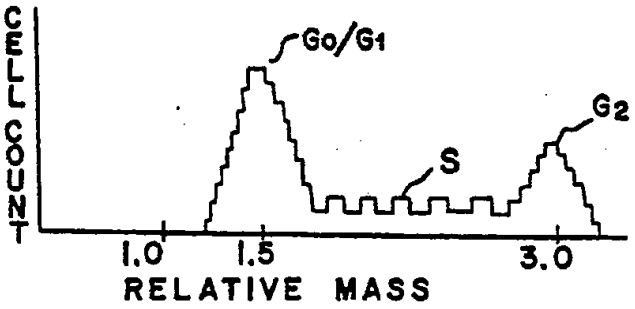


FIG. 9

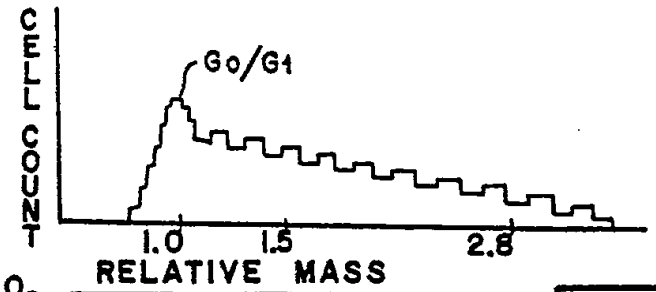


FIG. 10

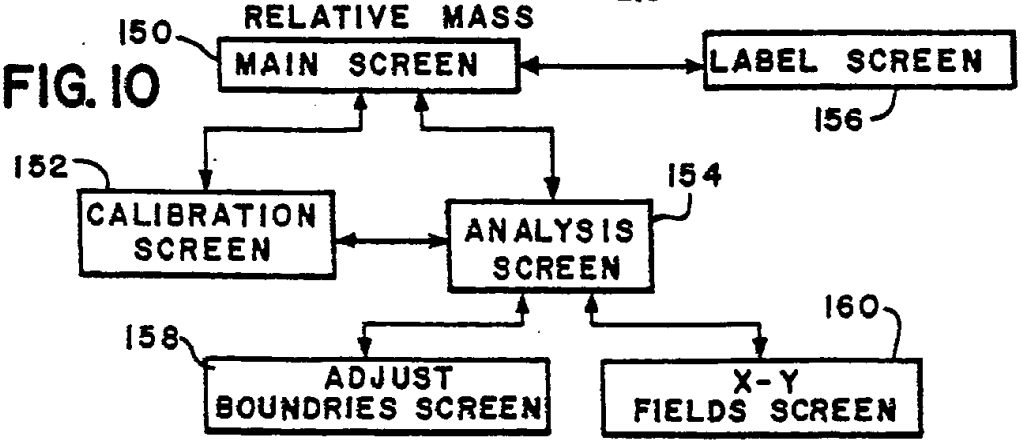


FIG. 11 MAIN SCREEN

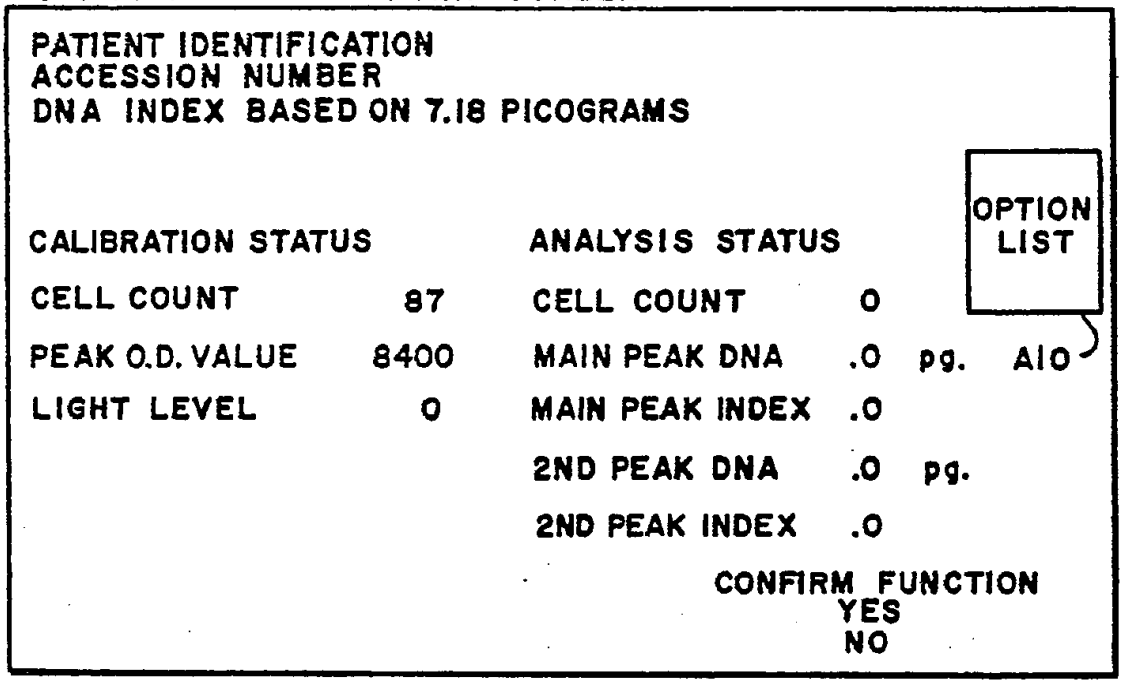


FIG. 12 CALIBRATION SCREEN

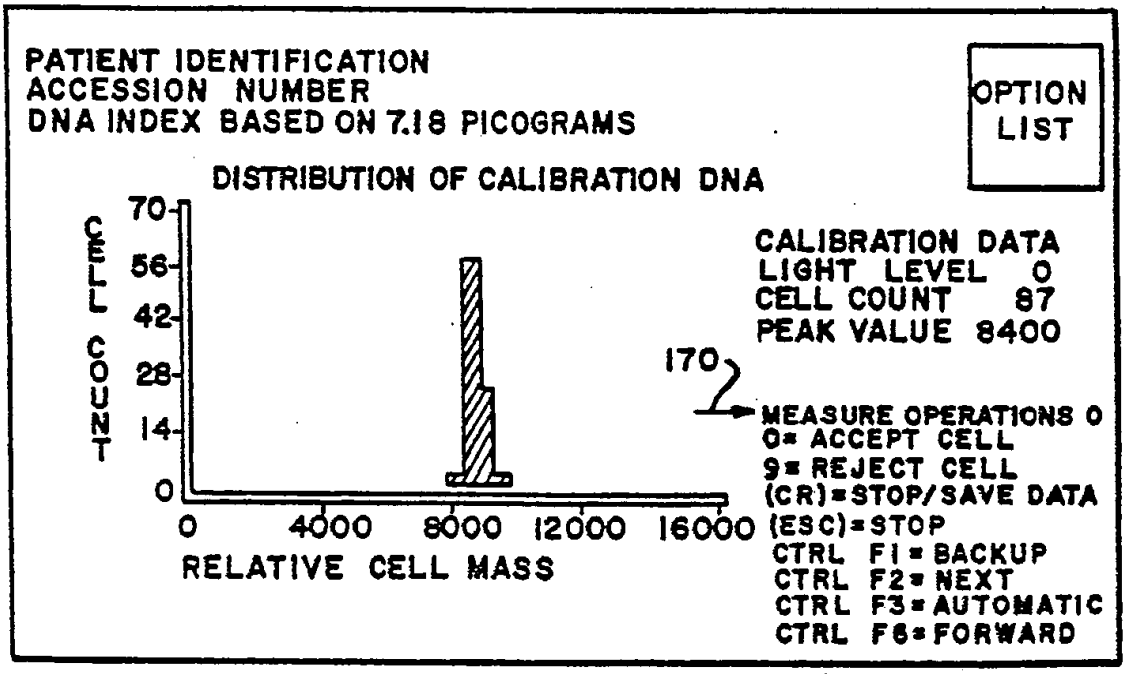


FIG. 13

ANALYSIS SCREEN

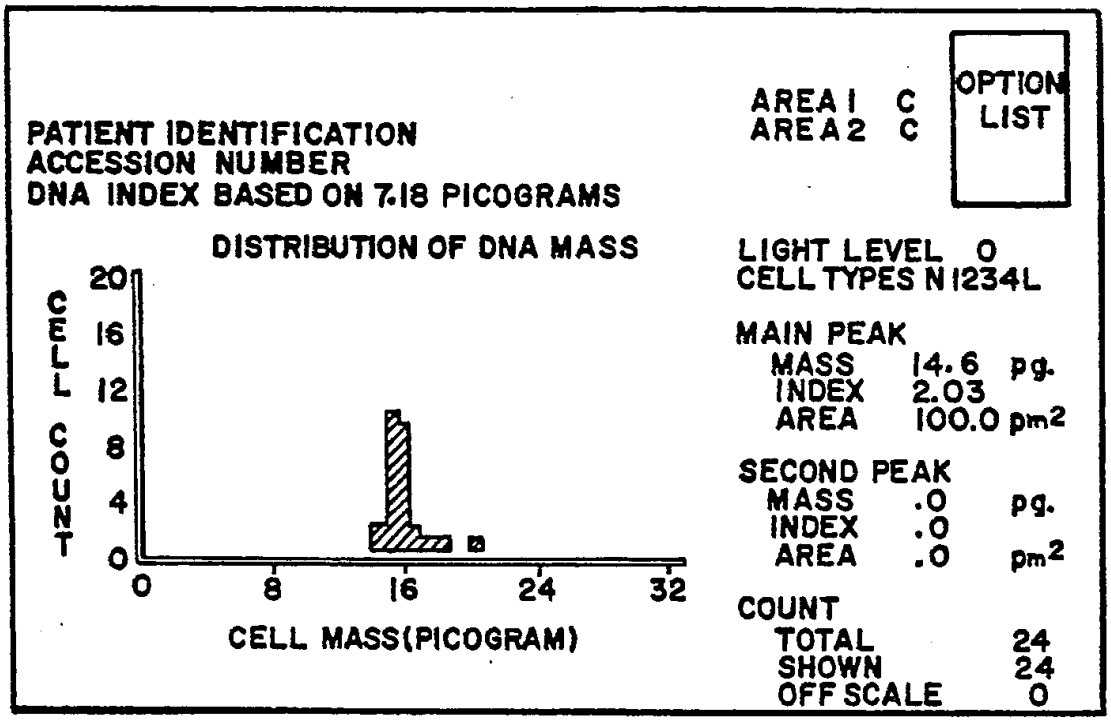


FIG. 14

X,Y FIELD COORDINATES SCREEN

FIELD#	X	Y	FIELD#	X	Y	FIELD#	X	Y
1	1243	0218						
2	1236	0210						
3	1235	0039						
4	1183	0078						

OPTION LIST

FIG. 15

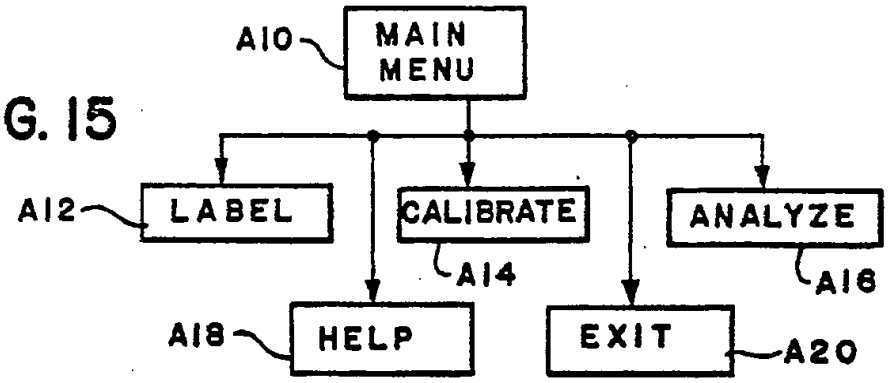


FIG. 16

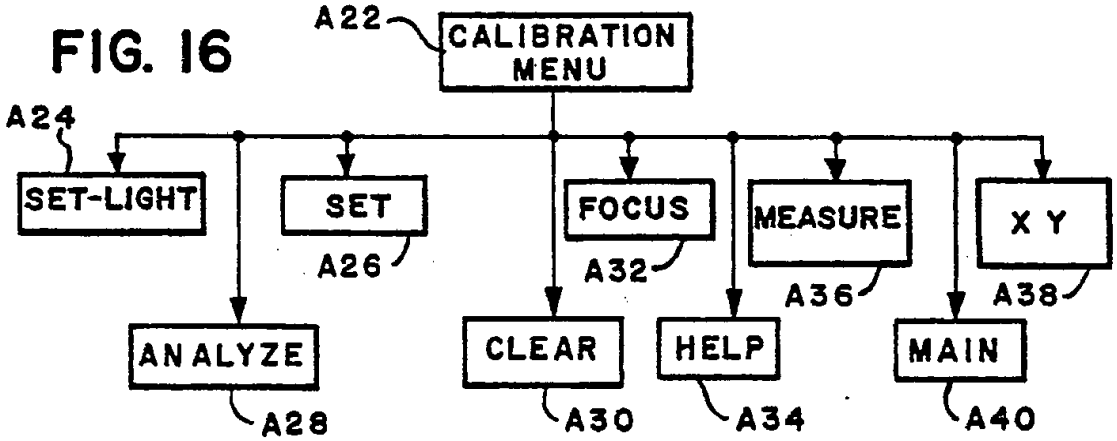


FIG. 17

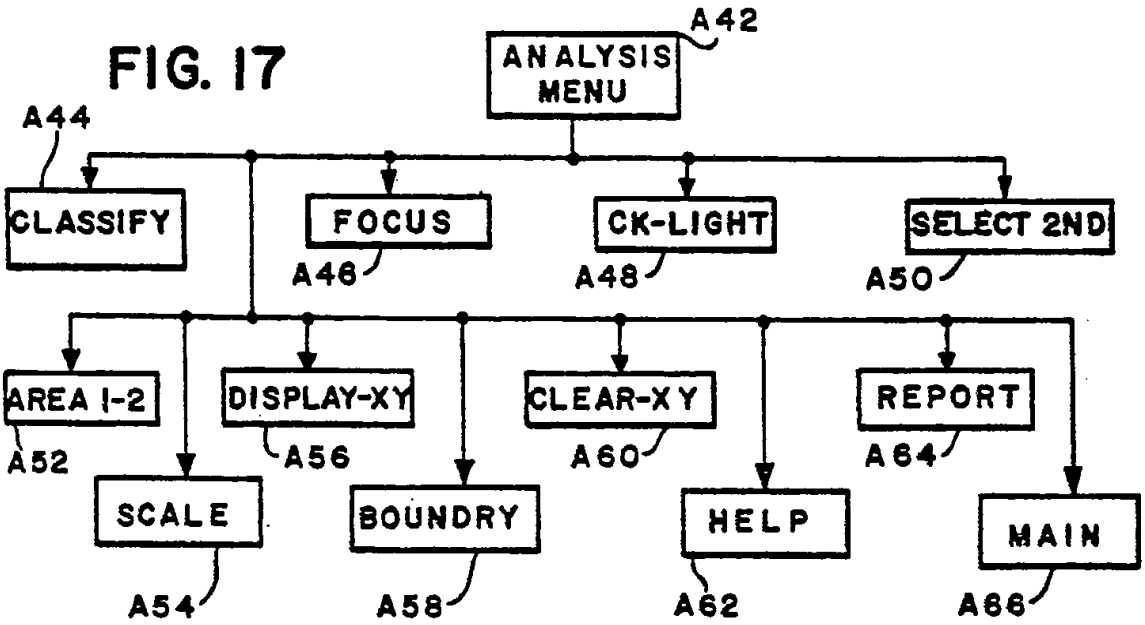


FIG. 18

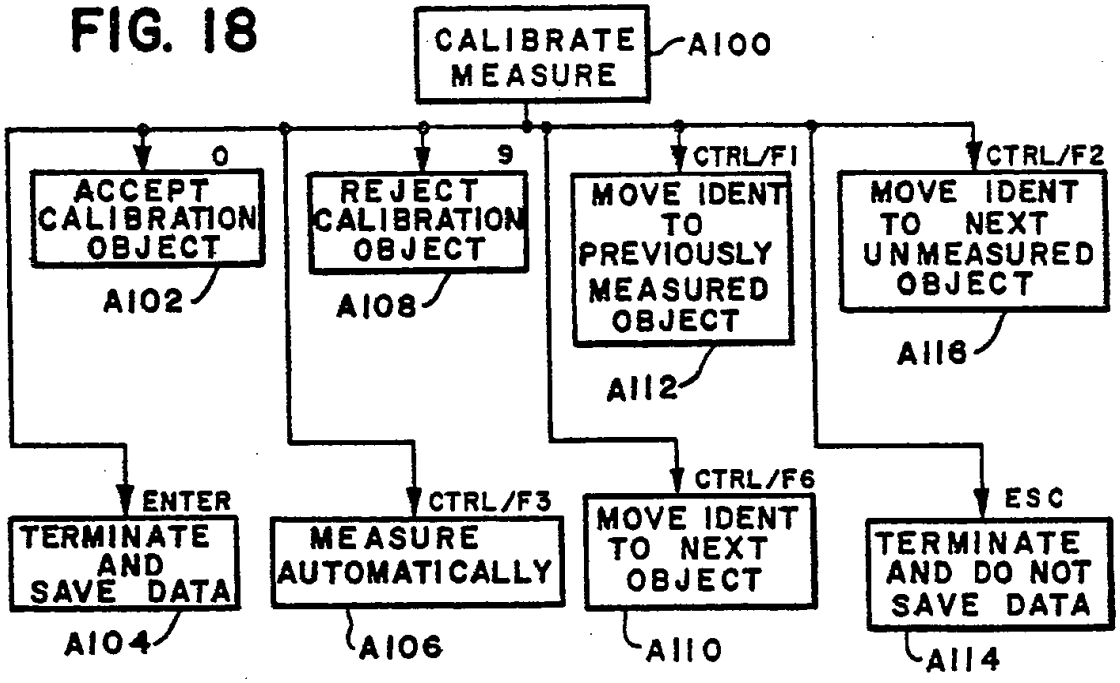

CALIBRATE MEASURE
A100
0
ACCEPT CALIBRATION OBJECT
A102
9
REJECT CALIBRATION OBJECT
A108
CTRL/F1
MOVE IDENT TO PREVIOUSLY MEASURED OBJECT
A112
CTRL/F2
MOVE IDENT TO NEXT UNMEASURED OBJECT
A116
ENTER
TERMINATE AND SAVE DATA
A104
CTRL/F3
MEASURE AUTOMATICALLY
A106
CTRL/F6
MOVE IDENT TO NEXT OBJECT
A110
ESC
TERMINATE AND DO NOT SAVE DATA
A114

FIG. 19

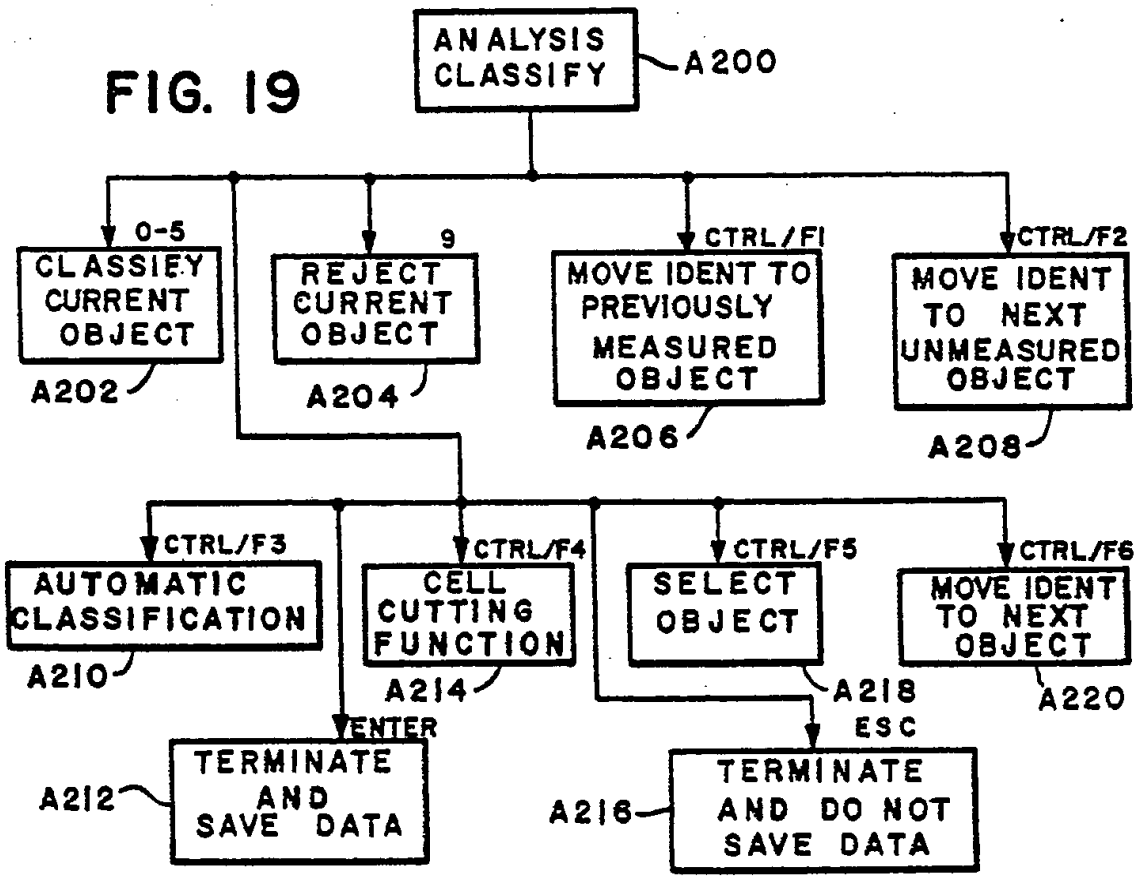

ANALYSIS CLASSIFY
A200
0-5
CLASSIEY CURRENT OBJECT
A202
9
REJECT CURRENT OBJECT
A204
CTRL/F1
MOVE IDENT TO PREVIOUSLY MEASURED OBJECT
A206
CTRL/F2
MOVE IDENT TO NEXT UNMEASURED OBJECT
A208
CTRL/F3
AUTOMATIC CLASSIFICATION
A210
CTRL/F4
CELL CUTTING FUNCTION
A214
CTRL/F5
SELECT OBJECT
A218
CTRL/F6
MOVE IDENT TO NEXT OBJECT
A220
ENTER
A212
TERMINATE AND SAVE DATA
ESC
A216
TERMINATE AND DO NOT SAVE DATA

FIG. 20

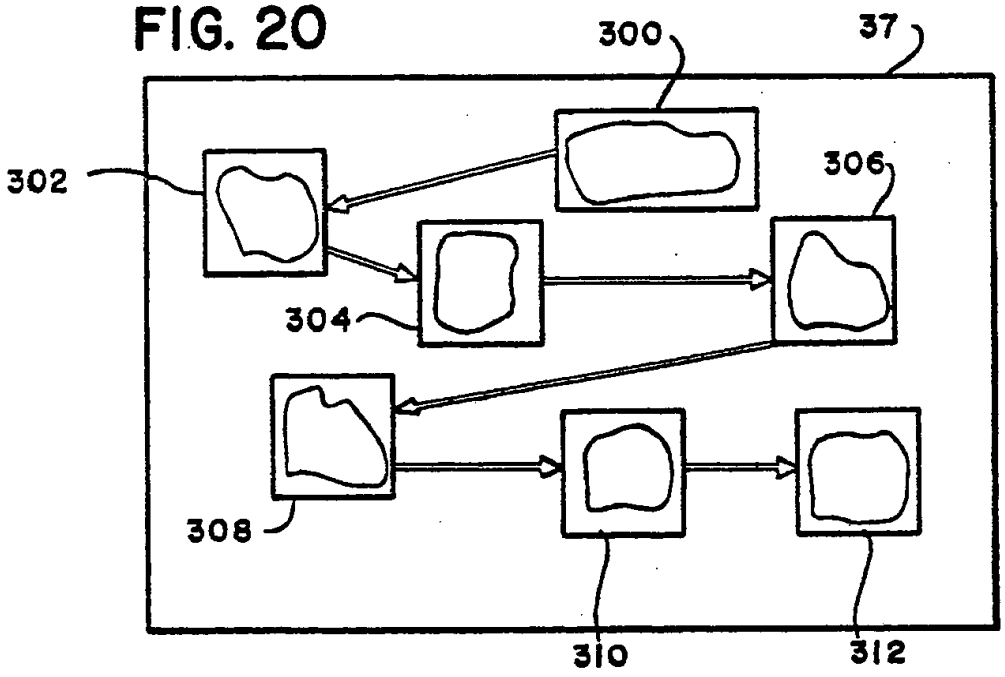


FIG. 21

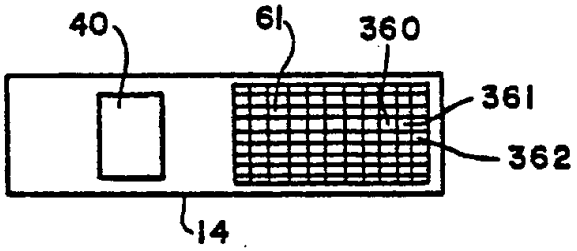
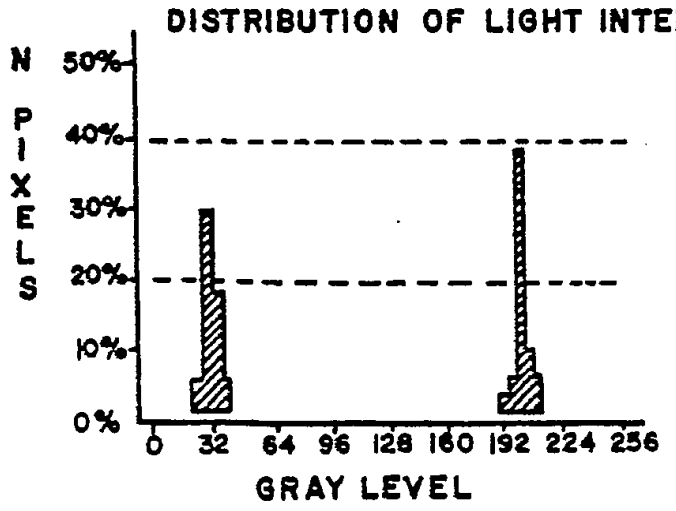


FIG. 22



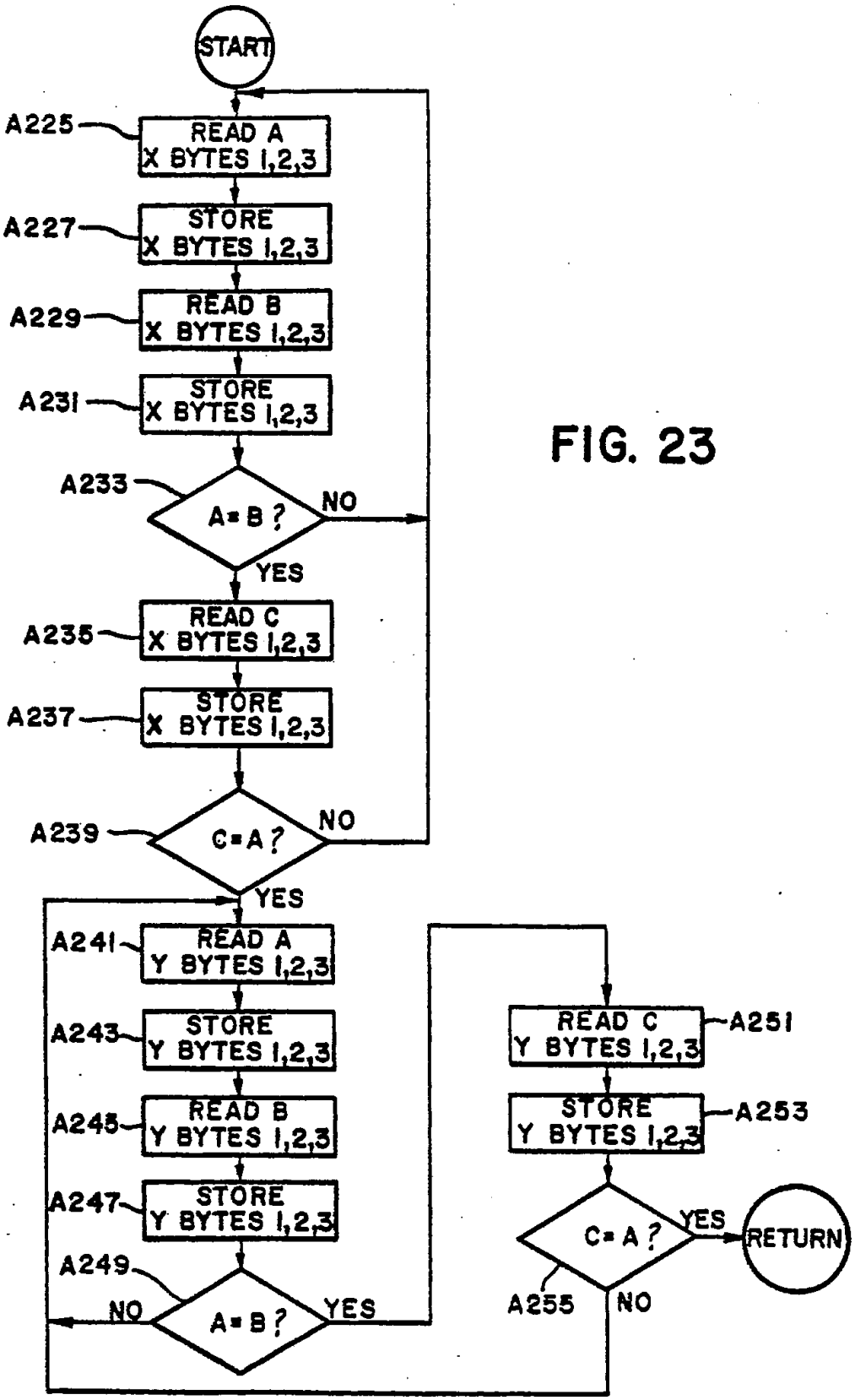
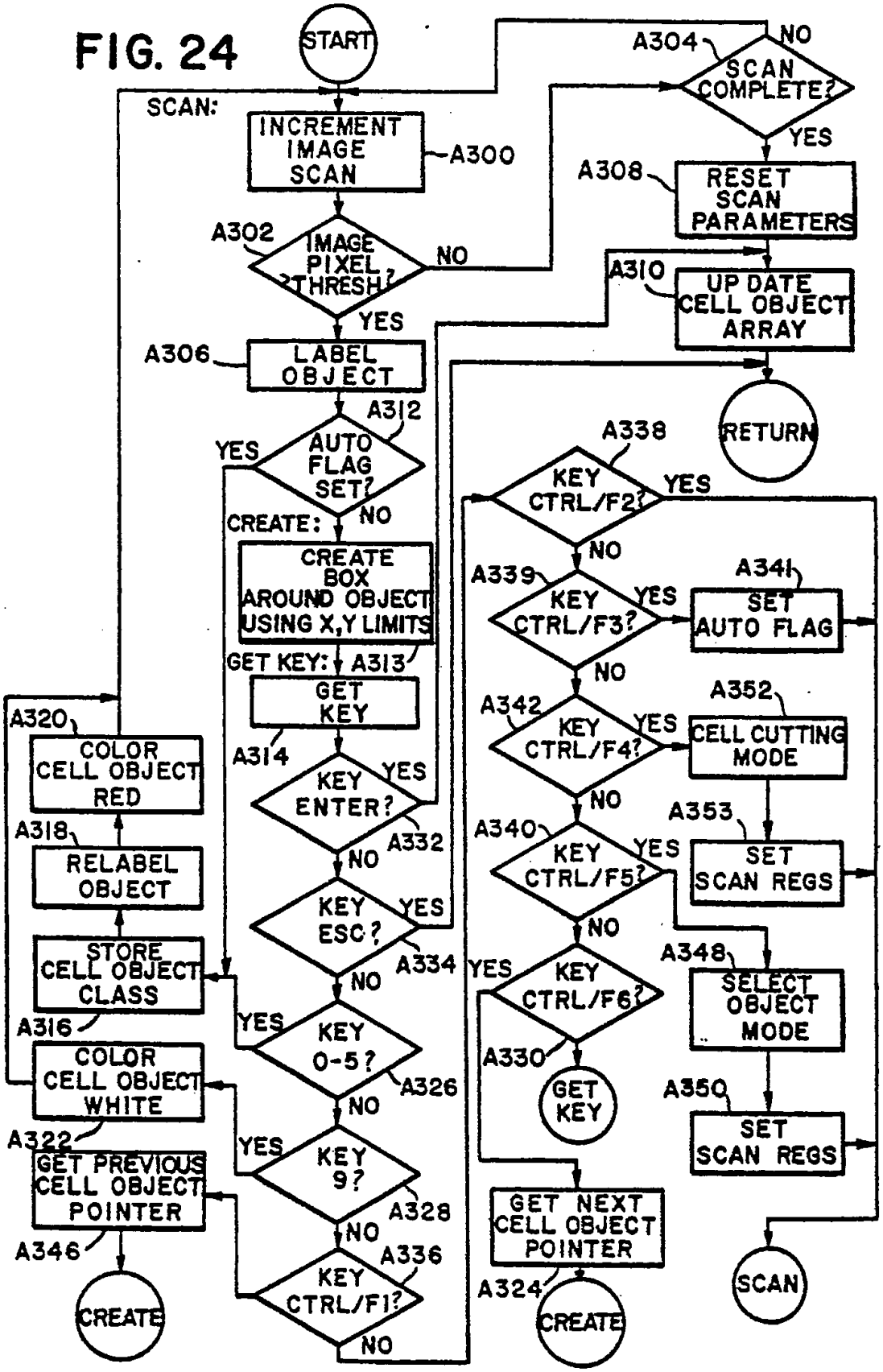

START
A225
READ A
X BYTES 1,2,3
A227
STORE
X BYTES 1,2,3
A229
READ B
X BYTES 1,2,3
A231
STORE
X BYTES 1,2,3
A233
A=B ?
NO
YES
A235
READ C
X BYTES 1,2,3
A237
STORE
X BYTES 1,2,3
A239
C=A ?
NO
YES
A241
READ A
Y BYTES 1,2,3
A243
STORE
Y BYTES 1,2,3
A245
READ B
Y BYTES 1,2,3
A247
STORE
Y BYTES 1,2,3
A249
A=B ?
NO
YES
A251
READ C
Y BYTES 1,2,3
A253
STORE
Y BYTES 1,2,3
C=A ?
YES
RETURN
A255
NO

FIG. 23

FIG. 24


START
SCAN:
INCREMENT IMAGE SCAN
A300
A302
IMAGE PIXEL >THRESH?
NO
YES
A306
LABEL OBJECT
A312
AUTO FLAG SET?
YES
NO
CREATE:
CREATE BOX AROUND OBJECT USING X,Y LIMITS
GET KEY: A313
GET KEY
A314
KEY ENTER?
YES
A332
NO
KEY ESC?
YES
A334
NO
KEY 0-5?
YES
A326
NO
KEY 9?
YES
A328
NO
KEY CTRL/F1?
A336
NO
A304
SCAN COMPLETE?
NO
YES
A308
RESET SCAN PARAMETERS
A310
UP DATE CELL OBJECT ARRAY
RETURN
A338
KEY CTRL/F2?
YES
NO
A339
KEY CTRL/F3?
YES
A341
SET AUTO FLAG
NO
A342
KEY CTRL/F4?
YES
A352
CELL CUTTING MODE
NO
A340
KEY CTRL/F5?
YES
A353
SET SCAN REGS
NO
KEY CTRL/F6?
YES
A348
SELECT OBJECT MODE
A330
GET KEY
A350
SET SCAN REGS
GET NEXT CELL OBJECT POINTER
A324
CREATE
SCAN
A320
COLOR CELL OBJECT RED
A318
RELABEL OBJECT
STORE CELL OBJECT CLASS
A316
COLOR CELL OBJECT WHITE
A322
GET PREVIOUS CELL OBJECT POINTER
A346
CREATE

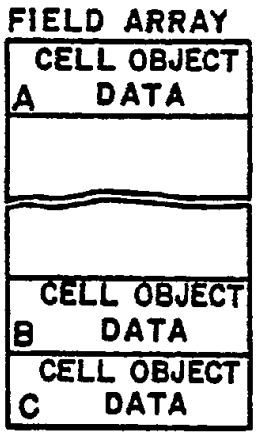


FIG. 24A

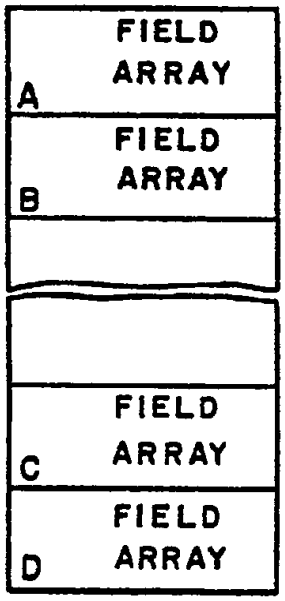


FIG. 24B

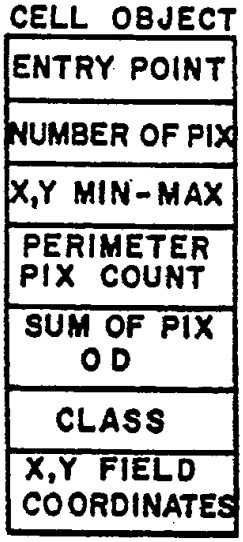


FIG. 24C

INTERNATIONAL SEARCH REPORT

International Application No PCT/US86/02409

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (4): G06K 9/00; G01N 21/00; G06F 15/42; G02B 21/26
U.S. Cl. 382/6; 356/39; 364/413; 350/531

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	382/6; 250/311; 350/507, 529, 531, 534, 535, 536; 356/39, 40; 358/107; 377/10; 364/413, 416

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [6]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X	JP, A, 59-88716 (TOSHIBA K K) 22 May 1984, see abstract.	1,3
X — Y	US, A, 4,129,854 (SUZUKI ET AL) 12 December 1978, see the entire document.	1,2,5,15-16, 18-19,40-49 / 6,7,41-43, 53-55,72
X — Y	US, A, 4,207,554 (RESNICK ET AL) 10 June 1980, see the entire document.	22-39 / 53-55,58-59
Y	US, A, 4,174,178 (OUCHI ET AL) 13 November 1979, see column 5, line 39 to column 6, line 38.	4,56-57
Y	US, A, 4,408,231 (BUSHAW ET AL) 04 October 1983, see column 3, lines 32 to 60.	44-46
Y,P	US, A, 4,592,089 (HARTMAN) 27 May 1986, see column 5, line 15 to column 6, line 48 and column 9, lines 9 to 52.	20-21,50-52, 61,65

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
06 FEBRUARY 1987	18 FEB 1987
International Searching Authority [1]	**Signature of Authorized Officer [10]**
ISA/US	LEO H. BOUDREAU, PRIMARY EXAMINER

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
Y	US, A, 3,297,879 (MEYER) 10 January 1967, see column 2, line 65 to column 3, line 58.	60
Y	US, A, 4,513,438 (GRAHAM ET AL) 23 April 1985, see column 5, lines 32 to 45.	62, 78-94
Y	US, A, 4,175,860 (BACUS) 27 November 1979, see column 4, line 31 to column 5, line 13.	63-71,75